

# **Stock Creek Watershed Restoration Plan**

**November 2007**

## **Acknowledgements**

*This plan was developed from the cooperative efforts of the partner organizations that make up the Stock Creek Initiative. It is intended to guide efforts to restore Stock Creek and its tributaries to fully supporting status for all designated uses, and to protect public health and well-being by addressing water quality issues that accompany agricultural and urban land uses. This restoration plan conforms to EPA Section 319 watershed plan guidelines and addresses each of the nine required components identified by EPA as critical for achieving improvements in water quality.*

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**\* Denotes an EPA-required component for watershed plans (EPA, 2003)**

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## List of Acronyms

BMP	Best Management Practice
CFU	Colony Forming Units
GIS	Geographic Information System
HUAP	Heavy Use Area Protection
HUC	Hydrological Unit Code
IBI	Index of Biotic Integrity
IPSI	Integrated Pollutant Source Identification
MPC	Knoxville-Knox County Metropolitan Planning Commission
MS4	Municipal Separate Storm Sewer System
NGVD	National Geodetic Vertical Datum
NPDES	National Pollutant Discharge Elimination System
NWI	National Wetlands Inventory
NPS	Nonpoint Source
NRCS	Natural Resources Conservation Service
PLM	Pollution Loading Model
SCWI	Stock Creek Watershed Initiative
TDEC	Tennessee Department of Environment and Conservation
TMDL	Total Maximum Daily Load
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
TVA	Tennessee Valley Authority
UT	University of Tennessee

## Executive Summary

The Stock Creek Watershed (HUC TN-060102010108) is located in the 638-square-mile Fort Loudoun Watershed of East Tennessee. Its 21 square miles lie almost entirely within the southern portion of Knox County and drain into Stock Creek, a tributary of the Little River.

The Stock Creek Watershed is primarily rural in character with two-thirds of its landscape in rolling pasture and forest. As a result of the popularity of the watershed's pastoral environment, its northern portion is beginning to urbanize and is characterized by a growing number of subdivisions and commercial developments, separated by farms and forested areas. Stock Creek's population grew by 12.4% between 1990 and 2000 and is expected to increase to 7,361 by the year 2030, an additional increase of 28%.

While Stock Creek's natural beauty has attracted more people to the area, its water quality is far from pristine. Nearly all of Stock Creek and its major tributaries are on the State of Tennessee's 303(d) list of impaired streams. Causes of impairment include *E. coli* from animal and human waste and loss of biological integrity due to siltation and physical substrate habitat alteration. Pollution sources include failed septic systems, agricultural practices, commercial development, and discharges from Knox County's NPDES-permitted Municipal Separate Storm Sewer System (MS4).

Total Maximum Daily Load (TMDL) targets for Siltation and Habitat Alteration and for Pathogens have been developed by the Tennessee Department of Environment and Conservation (TDEC) and have been approved by the Environmental Protection Agency (EPA) for the Fort Loudoun Watershed. The TMDLs require an 88% reduction in pathogens and a 35.3% reduction in sediment in Stock Creek.

Stock Creek's water quality problems have not gone unnoticed by local organizations, governmental agencies, and area residents. Through cooperative efforts, in particular the efforts of the partners that comprise the Stock Creek Watershed Initiative (SCWI), a consortium of agencies, universities, and utilities that formed in 2002, a great amount of information about the watershed has already been compiled and the essential groundwork has been laid for a multi-pronged approach to restoration, of which this plan is a key component.

This watershed restoration plan (WRP) was developed to provide a comprehensive plan for restoring the water quality of Stock Creek and its tributaries so they can fully support their designated uses and can be removed from the 303(d) list. Model results from an Integrated Pollution Source Identification (IPSI) study performed by the Tennessee Valley Authority (TVA) have been used to determine land use and priority areas for restoration activities. The SCWI partners expect it will take 15 years to reduce pathogens and sediment to the level required by the TMDLs.

This plan details Phase I of that comprehensive plan. It contains details for a five-year strategy to reduce *E. coli* concentrations by 29% by addressing failed septic systems and livestock pathogen sources. Best Management Practices (BMPs), such as stream buffer restoration and the improvement of pasture conditions, will be implemented to reduce livestock pathogens. These BMPs have the added benefit of reducing total suspended solids (TSS), another cause of impairment to the creek.

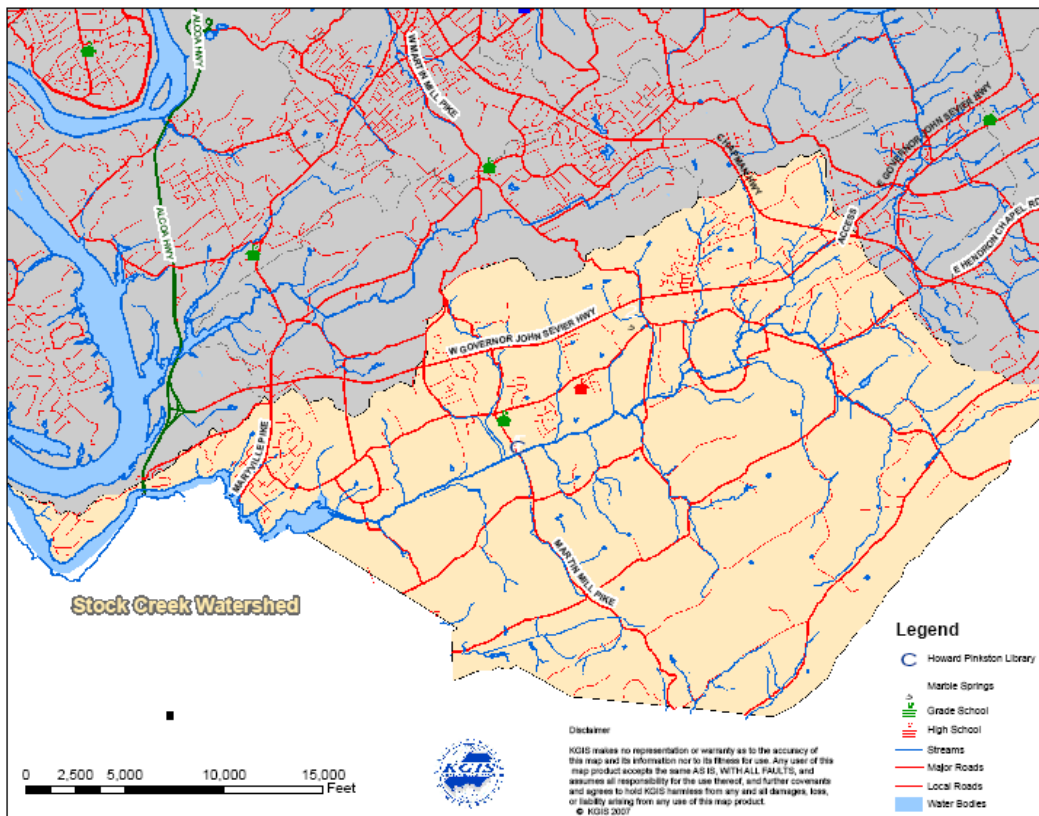
Periodically, an assessment of the biological community of the creek will be conducted to determine if the reduction goal is adequate for stream recovery. Adjustments to the strategy will be made as needed and, during Year Five, a detailed plan for Phase II will be designed based on an evaluation of accomplishments and monitoring results.

This plan follows EPA's Section 319 watershed plan guidelines and addresses each of the nine required components. Sections of this plan that specifically address one of these nine components are indicated with an \* after the section title.

# 1.0 Introduction

## Background

The Stock Creek Watershed (HUC TN-060102010-108) in East Tennessee drains an area of approximately 21 square miles in the 638-square-mile Fort Loudoun Watershed (Figure 1). Located almost entirely within the southern part of Knox County, with a portion in Blount County, the Stock Creek Watershed drains into Stock Creek, which flows for 24 miles to the Little River.



**Figure 1 Map of Beaver Creek Watershed**

With almost two-thirds of its landscape consisting of rolling pasture or forest, the Stock Creek Watershed retains a largely rural character, as it begins to urbanize. Despite the Watershed's natural beauty, however, Stock Creek's water quality is poor. The Tennessee Department of Environment and Conservation (TDEC) has determined that its water quality is impaired due to high bacteria levels, siltation, and habitat alteration. (TDEC, 2006a) This means the Creek's quality is too poor to support its designated uses – fish and other aquatic life, irrigation, livestock watering and wildlife, and recreation.



According to TDEC, a significant reduction in pathogens and siltation will be required to restore the water quality of Stock Creek. Specifically, the Fort Loudoun Total Maximum Daily Load (TMDL) established by TDEC requires an 88.0% reduction in pathogens (Table 1) and a 35.3% reduction in sediment (Table 2) in Stock Creek.

**Table 1 TDEC TMDL for Pathogens in Stock Creek**

HUC-12 Subwatershed (06010201__) or Drainage Area	Impaired Waterbody	Impaired Waterbody ID	TMDL	WLAs		LAs	
				Leaking Collection Systems <sup>a</sup>	MS4s <sup>b</sup>	Precipitation Induced Nonpoint Sources	Other Direct Sources <sup>c</sup>
			[% Red.]	[cts./day]	[% Red.]	[% Red.]	[cts./day]
0108	Grandview Branch	TN06010201066 – 0300	88.0	NA	88.0	88.0	0
	High Bluff Branch	TN06010201066 – 0600					
	Stock Creek	TN06010201066 – 1000 & 2000					
	Gun Hollow Branch	TN06010201066 – 1200					

**Table 2 TDEC TMDL for Sediment in Stock Creek**

TMDLs, WLAs for MS4s and Construction Storm Water Sites, LAs for Nonpoint Sources (Cont.):

HUC-12 Subwatershed (06010201__)	Waterbody ID	Waterbody Impaired by Siltation/ Habitat Alteration	Level IV Ecoregion	TMDL (Required Overall Load Reduction	Required Load Reduction	
				[%]	WLA (MS4s and Const. SW)	LA (Nonpoint Sources)
					[%]	[%]
0108	06010201066_0100	Casteel Branch	67h	35.3	38.6	38.6
	06010201066_0200	Twin Branch				
	06010201066_0500	McCall Branch				
	06010201066_1000	Stock Creek				

## Partnerships and Accomplishments

Significant progress has been made in the areas of building partnerships, educating citizens, assessing conditions, and identifying pollution sources in recent years. Cooperative efforts to address water quality issues in the Stock Creek Watershed originated with the Water Quality Forum, an organization formed in 1990 to address water quality and water quantity issues in Knoxville and surrounding counties.

In 2002, under the direction of the Water Quality Forum, a consortium of 12 local, state, and federal agencies formed the Stock Creek Watershed Initiative (SCWI). Its mission is to bring together public and private institutions to implement a program to restore Stock Creek back to a healthy stream that is fully supporting its designated uses by implementing restoration practices and promoting sound land use planning. The SCWI partners are:

- City of Knoxville, Tennessee
- Knox County Soil Conservation District
- Knox County, Tennessee
- Knox-Chapman Utility District
- Knoxville/Knox County/Knoxville Utility Board Geographical Information System

- Knoxville-Knox County Metropolitan Planning Commission
- Little River Water Quality Forum
- Little River Watershed Association
- Natural Resource Conservation Service
- South Doyle Homeowner's Association
- TDEC - Division of Water Pollution Control, Knoxville EAC
- Tennessee Valley Authority
- Tennessee Water Resources Research Center
- United States Geological Survey
- University of Tennessee

The SCWI partners have made some significant accomplishments that will serve as a good foundation for further restoration work:

**Technical Subcommittee:**

- Developed IPSI model to target pollution sources and prioritize areas
- Identified IPSI bacteriological sources

**Education Subcommittee:**

- Developed and implemented communication plan
- Developed and distributed brochure
- Worked with AmeriCorps members to educate area high school students on improving water quality
- Organized public meetings

**Watershed Planning:**

- Received Non-Point Source Program (Section 319(h)) Grant in order to develop a Watershed Action Plan with public involvement

**Sewer Line Extension Studies/Outreach:**

- Developed partnership with South Knox Utility District
- Developed plan for a four-phase sewer extension project into septic failure areas
- Received Community Development Block Grant for Phases One and Two

## **Purpose of this Plan**

Though water quality is poor in the Stock Creek Watershed, interest in improving water quality has increased in recent years. While the watershed is still largely rural, wise land use decisions and proper planning hold great promise for restoring and protecting Stock Creek so that future generations may take pleasure in it as have those in the past.

This Watershed Action Plan proposes to build on that growing interest in water quality in the Stock Creek Watershed by combining the technical capabilities and resources of multiple agencies and the private sector to promote the use of best management practices (BMPs) that will minimize impacts on water resources. Pathogen and sediment from agricultural sources can frequently be addressed by the same BMPs. Modeling (see Section 3) indicates that sediment loading reduction goals will be achieved in Stock Creek before pathogen loading reduction goals are reached, with no sediment-specific practices required.

This plan follows the current EPA Section 319 watershed plan guidelines and addresses each of the nine required components (USEPA, 2003). It serves as a guide to the Stock Creek Initiative partners and outlines their actions to restore water quality in the Stock

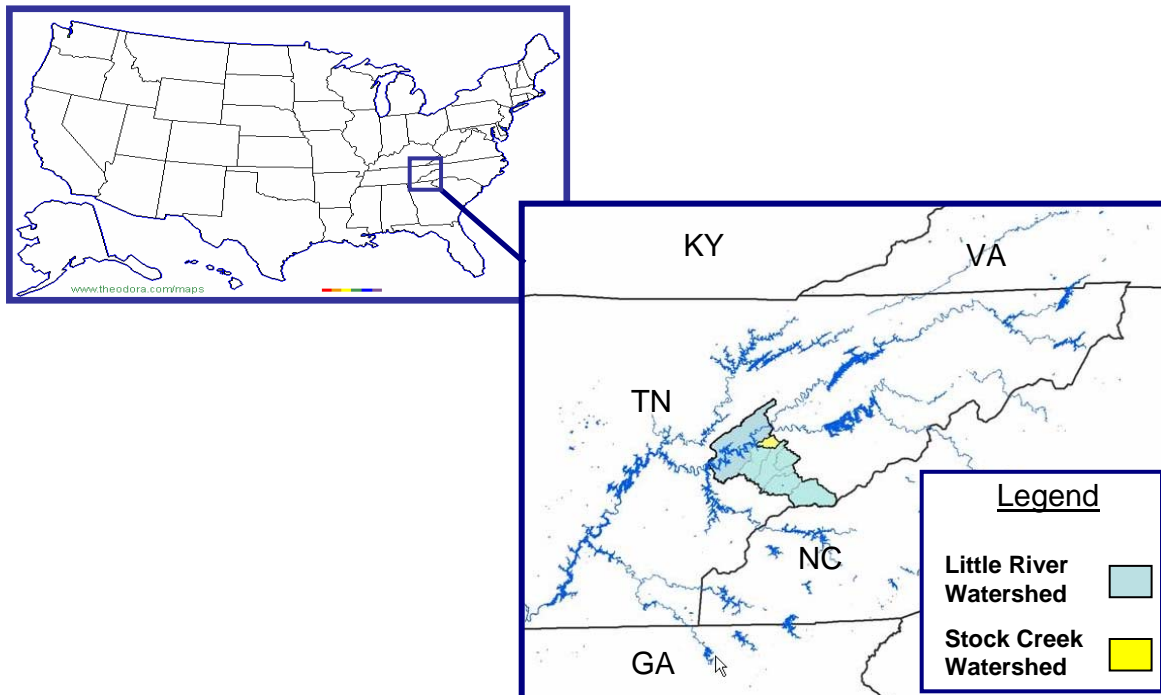
Creek Watershed. It also contains details for a 5-year Phase I effort toward this end. Periodically, efforts and results will be re-evaluated and adapted as necessary to achieve goals. At completion, success of the restoration plan will be measured and evaluated through data results.

## 2.0 Description of the Watershed

### 2.1 Physical Characteristics

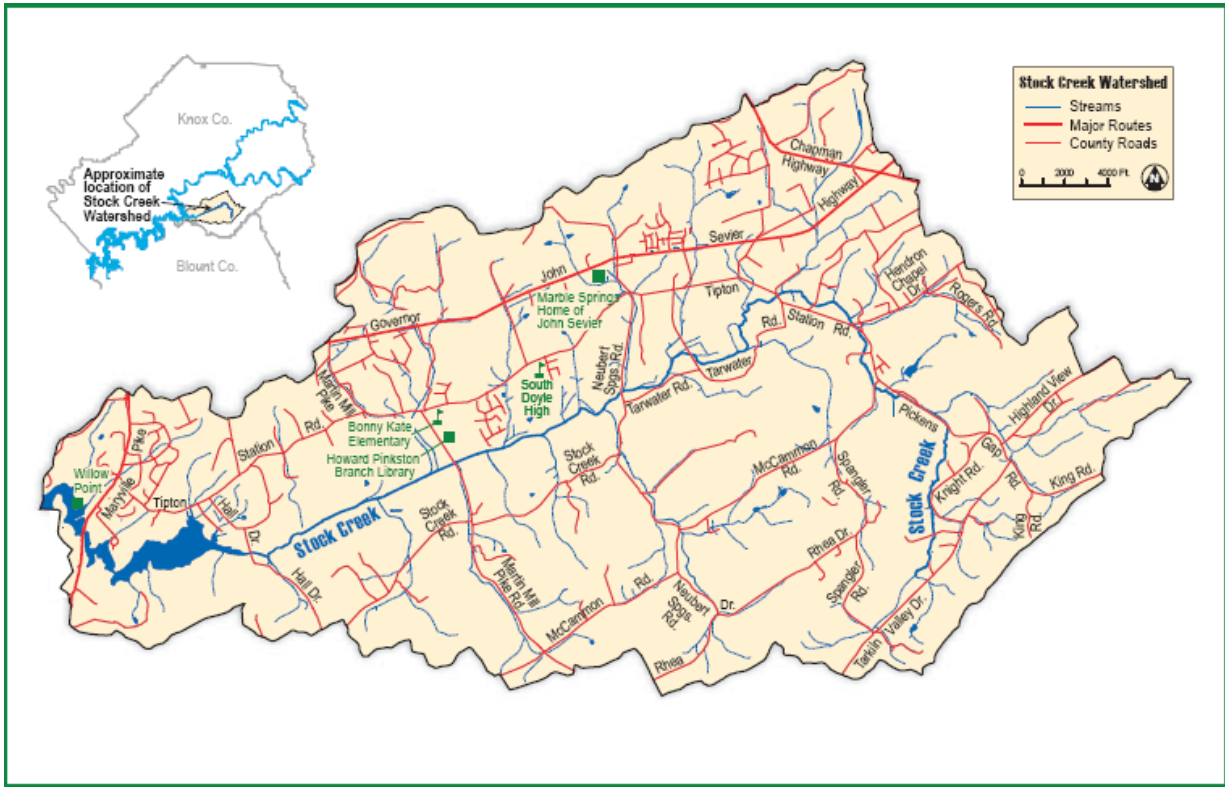
#### Location

The Stock Creek Watershed is a subwatershed of the Little River located in the eastern portion of the State of Tennessee in the Southeastern United States. (Figure 2) The watershed area drains into the Fort Loudoun Reservoir which is part of the Tennessee River system.



**Figure 2 Map of Stock Creek Watershed Location within the Continental U.S.**

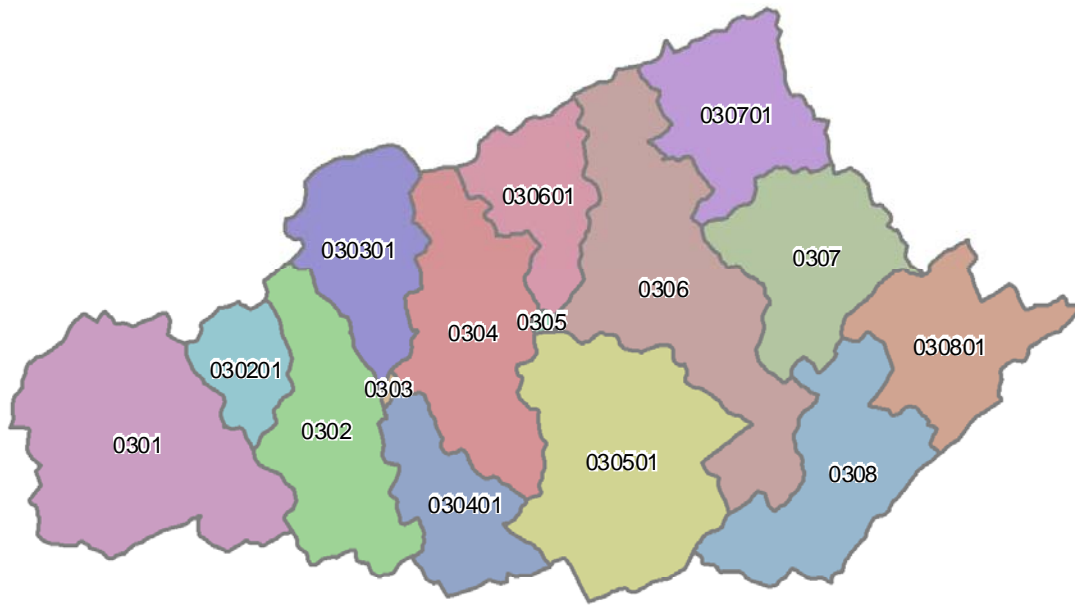
A large portion of the Stock Creek watershed is located within the southern Knoxville Tennessee metropolitan area approximately 36 miles west of the Great Smoky Mountains National Park and the Appalachian Mountains. (Figure 3)



**Figure 3 Map of Stock Creek Watershed Location within Knox County**

**Subwatersheds**

The Stock Creek Watershed has been divided into 13 subwatersheds and 15 sampling sites (Are 0303 and 0305 considered subwatersheds, watershed IDs, sampling sites, or what? Table below is confusing since it contains a listing of 15 “subwatersheds”), as shown by the various colors and numbers, respectively, in Figure 4.



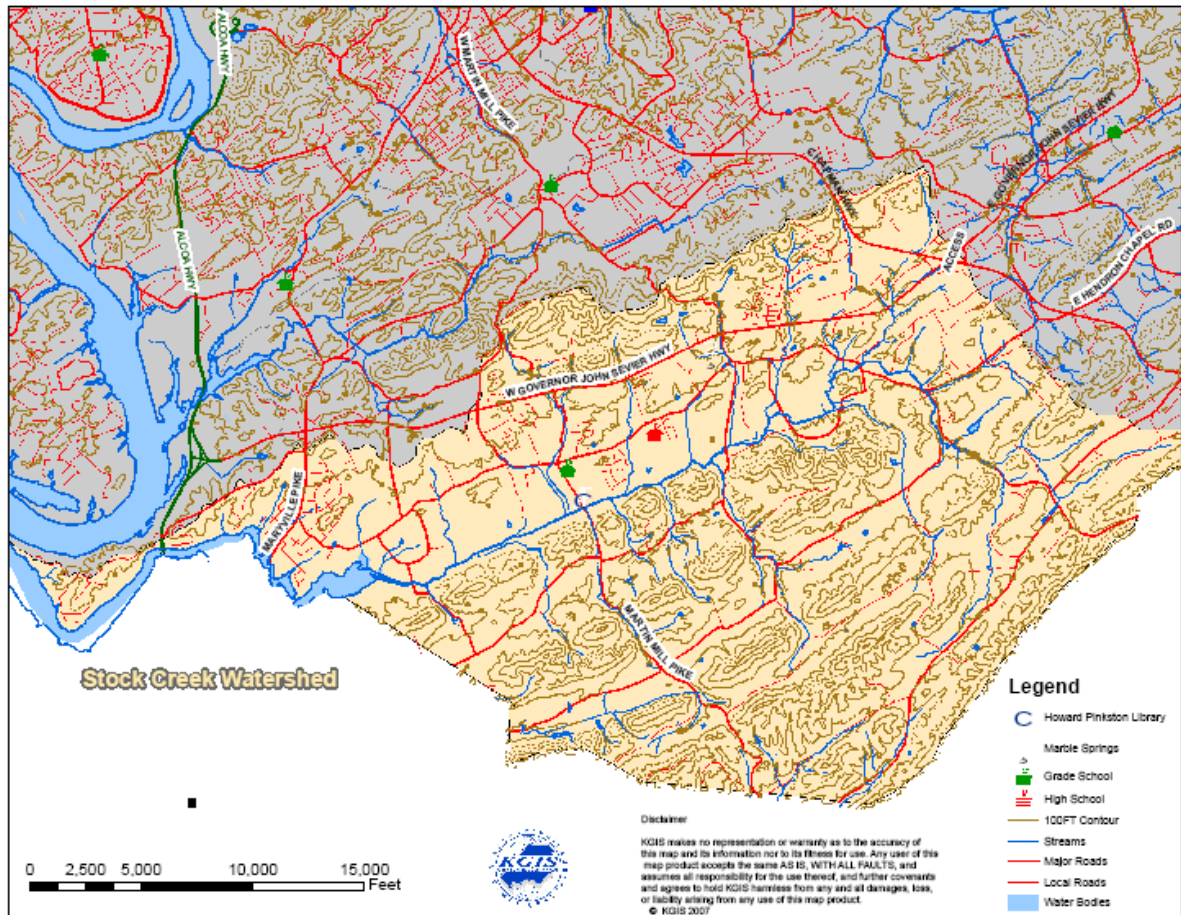
<b>Watershed ID</b>	<b>Subwatershed Name*</b>
0301	Stock Cr., Mouth to Casteel Br.
0302	Stock Cr., Casteel Cr. to Twin Cr. (includes Gun Hollow, GH-1)
030201	Casteel Branch
0303	Stock Cr., Twin Cr. to unnamed tributary at SCM 3.2
030301	Twin Cr.
0304	Stock Cr., unnamed tributary at SCM 3.2 to unnamed tributary at SCM 4.4
030401	Unnamed tributary at SCM 3.2 (Martin Mill, MM-1)
0305	Stock Cr., unnamed tributary at SCM 4.4 to unnamed tributary at SCM 4.8
030501	Unnamed tributary at SCM 4.4 (Neubert Springs, NS-1)
0306	Stock Cr., unnamed tributary at SCM 4.8 to McCall Br. (includes Grandview (GV-1; SC-3; SC-4; and SB-1)
030601	Unnamed tributary at SCM 4.8 (Sevier Home, SH-1)
0307	Stock Cr., McCall Branch to unnamed tributary at SCM 8.1 (includes SC-5; SC-6; and High Bluff, HB-1)
030701	McCall Branch (MB-1)
0308	Stock Cr., unnamed tributary at SCM 8.1 to head (SC-7)
030801	Unnamed tributary at SCM 8.1 (Nichols Mountain, NM-1)

\* “SCM”=Stock Creek Mile, measured from the mouth

**Figure 4 Stock Creek Subwatersheds**

## Topography

The Stock Creek Watershed is located in the valley and ridge physiographic region on the western flanks of the Appalachian Mountains and is characterized by alternating northeast-southwest trending ridges of Paleozoic sedimentary rocks. The Stock Creek Watershed is underlain mainly by karstic carbonate rock. The ridges and valleys were formed by thrust faulting of the underlying bedrock early in the development of the landscape. The relief of the Stock Creek watershed is mainly rolling to hilly, but has numerous steep, fairly rugged ridges. (Figure 5)



**Figure 5 Topographic Map of the Stock Creek Watershed**

As a tributary to the Little River embayment, Stock Creek's main stem winds 24 miles through the mostly rural landscape of South Knox County. The approximate elevation of the outlet of the watershed is 820 ft NGVD (national geodetic vertical datum) and the headwater ridges have an elevation of approximately 1290 ft NGVD. (Gentry, 2006)

## Climate

Air temperature in Knoxville ranges from an average January low of 29° F to an average high of 87° F in July. In the average year, there are 48.2” of total rain, 9.9” of snow, and 128 wet days (NWS, 2006).

## Ecoregions

The Stock Creek Watershed is located in the Southern Appalachian Ridge and Valley Level IV Ecoregions of 67f, 67g, and 67h, which are described in Table 3. (TDEC, 2005)

**Table 3 Stock Creek Watershed Ecoregions**

Ecoregion ID	Name	Characteristics
67f	Southern Limestone/ Dolomite Valleys and Low Rolling Hills	Mostly low rolling ridges and valleys; soils vary in their productivity; predominantly limestone and cherty dolomite; springs and caves are relatively numerous.
67g	Southern Shale Valleys	Consists of lowlands, rolling valleys, and slopes and hilly areas that are dominated by shale materials; soils tend to be acid; springs and caves are relatively numerous.
67h	Southern Sandstone Ridges	Encompasses major sandstone ridges, but ridges also have areas of shale and siltstone; steep, forested ridges have narrow crests; springs and caves are relatively numerous; soils are typically stoney, sandy, and of low fertility; chemistry of streams flowing down the ridges can vary greatly depending on the geologic material.

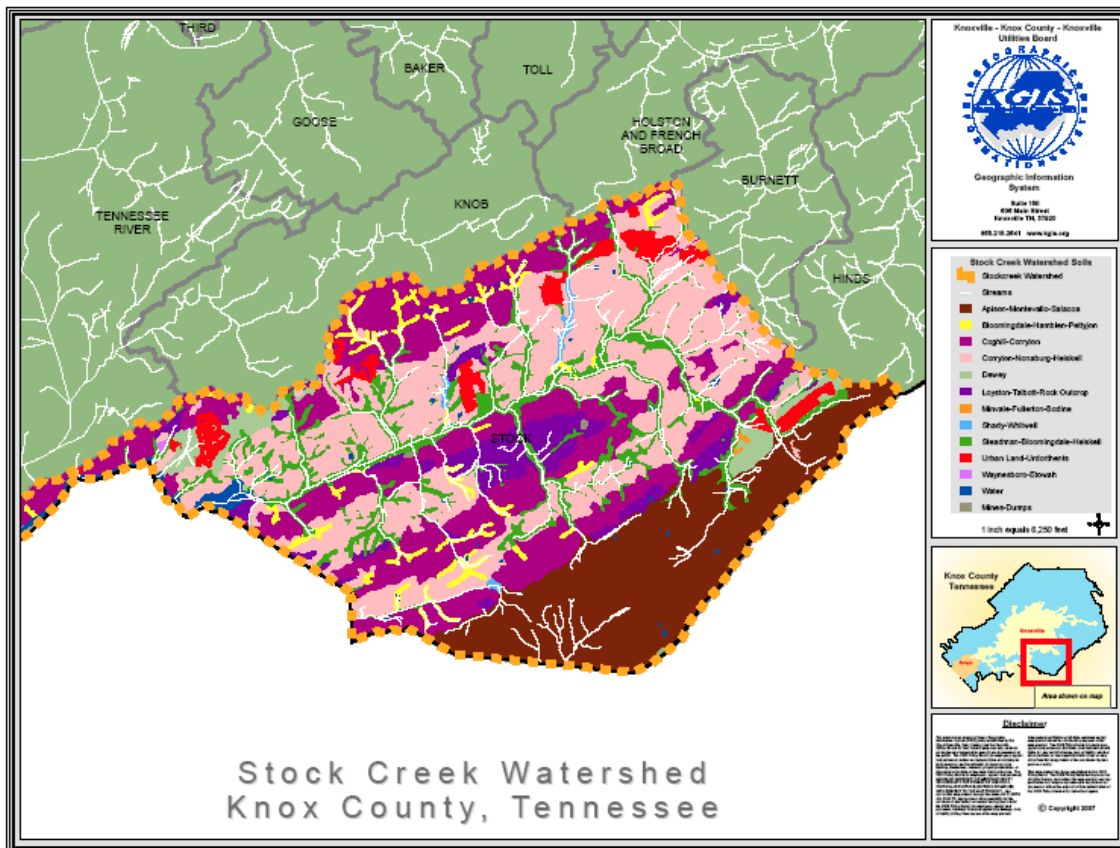
## Soils

Most of the soils in the Stock Creek Watershed have moderate to very severe limitations for most uses including building and construction, sanitary facilities, and even some agricultural uses. Some of these limitations include steepness of slope, limited depth to bedrock, clayey subsoils, and flooding and wetness. The prevalence, location, and use limitations of each type of soil found in the Stock Creek Watershed is shown in Table 4 and Figure 6. (KGIS, 2007)



**Table 4 Percentage of Each Soil Type in the Stock Creek Watershed**

General Soil Map Units	Area	Percent	Limitations
Apison-Montevallo-Salacoa	125787836.78520	18.32%	Very Limited: steepness of slope & depth to bedrock
Bloomington-Hamblen-Pettyjohn	14066528.47290	2.05%	Very Limited: flooding & wetness
Coghill-Corryton	188175322.25650	27.41%	Somewhat Limited: steepness of slope & clayey subsoil
Corryton-Nonaburg-Heiskell	186144253.74310	27.11%	Somewhat Limited: steepness of slope, clayey subsoil, depth to bedrock, wetness
Dewey	18764310.82670	2.73%	Somewhat Limited: steepness of slope & clayey subsoil
Loyston-Talbott-Rock Outcrop	32326916.37180	4.71%	Very Limited: steepness of slope, depth to bedrock, rock outcrops
Minvale-Fullerton-Bodine	314662.53750	0.05%	Somewhat Limited: steepness of slope, clayey subsoil, rock fragments
Shady-Whitwell	2520790.98560	0.37%	Very Limited: flooding & wetness
Steadman-Bloomington-Heiskell	88754717.08340	12.93%	Very Limited: flooding & wetness
Urban Land-Urdorthents	20049747.98730	2.92%	Very Limited: developed land
Waynesboro-Etowah	887260.65970	0.13%	Somewhat Limited: steepness of slope & clayey subsoil
Water	8479426.42130	1.24%	
Mines-Dumps	296138.32260	0.04%	
	686567912.45360	100.00%	



**Figure 6 Soil Types in the Stock Creek Watershed**

## Threatened or Endangered Species

The threatened or endangered species listed in Table 5 have previously been identified in the watershed. (TDEC, 2007)

**Table 5 Threatened and Endangered Species in the Stock Creek Watershed**

060102010108	Stock Creek		Federal Status	State Status	Global Rank	State Rank
<b>Bird</b>						
	<i>Accipiter striatus</i>	Sharp-shinned Hawk	No Status	D	G5	S3B
<b>Fish</b>						
	<i>Phoxinus tennesseensis</i>	Tennessee Dace		D	G3	S3

## Wetlands

The National Wetlands Inventory (NWI) provides a general idea of wetland occurrence. NWI identifies 633 acres of permanently flooded wetlands and farm ponds in the Stock Creek watershed, mostly smaller than 0.25 acres. (USFWS, 2007) Most of the area in the NWI estimate consists of the shallow reservoir embayment. The IPSI identifies an additional 8.3 acres of scrub/shrub wetland.

## 2.2 Human and Land Use Characteristics

### Cultural Resources

Early settlement in South Knox County occurred near the fertile lands along the rivers and streams, including Stock Creek. One settler, John Sevier, the first governor of Tennessee, was awarded a Revolutionary Land Grant of 640 acres in 1785. His property was located at the foot of Bays Mountain, an area where marble deposits had been found along large springs, thus Sevier named his farm "Marble Springs". Today it is a Tennessee state-owned historic site open to the public. (TDEC, 2005b)

### Human Population

According to the 1990 census, there were 5,110 residents in the Stock Creek watershed. By 2000, the population had increased to 5,744, an increase of 12.4%. The Metropolitan Planning Commission (MPC) projects a 0.83% per year escalation in population between the years 2000 and 2030. (KCMPC, 2007) This would increase the population within the watershed to 7,361 by the year 2030, an additional increase of 28%.

### Land Use

TVA developed an Integrated Pollutant Source Identification (IPSI) system for the Stock Creek Watershed to aid partners in addressing nonpoint source pollution. The IPSI system was designed as an aid to planning and implementing a watershed-based approach to pollution control and water quality improvements. IPSI is a geographic database generated by interpretation of low-altitude, color infrared, aerial photography obtained in late winter or early spring when leaves are off of the trees. Photography for the Stock

Creek watershed, as well as the rest of the Little River watershed, was obtained during the winter of 2002.

The Stock Creek Watershed is primarily rural in character with two-thirds of its landscape in rolling pasture and forest. As a result of the popularity of the watershed’s pastoral environment, its northern portion is beginning to urbanize and is characterized by a growing number of subdivisions and commercial developments, separated by farms and forested areas.

According to the IPSI data, 7.5 acres within the Stock Creek Watershed were identified as “disturbed,” most of which were probably areas under construction. Approximately 8.7% of the surface area of the watershed is impervious. (Table 6).

**Table 6 Percentage of Impervious Surface Area in Stock Creek Watershed**

<b>Subwatershed</b>	<b>Percent Impervious</b>
0301	11.6%
0302	7.2%
030201	9.1%
0303	12.3%
030301	9.5%
0304	10.6%
030401	5.6%
0305	6.4%
030501	4.1%
0306	7.3%
030601	9.2%
0307	9.3%
030701	20.7%
0308	6.0%
030801	6.2%
Stock Creek Total	8.7%

Just over half of the land is forested, with most of the rest of the land shared approximately equally between agricultural and residential uses. Commercial and industrial uses occupy only 4% of the land. (Figure 7)

Stock Creek land use/land cover

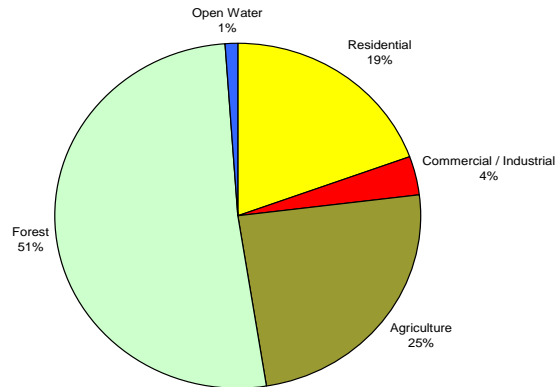


Figure 7 Stock Creek land use/land cover

Table 7 Stock Creek Land Use/Land Cover in Acres by Watershed

Watershed ID	Residential	Commercial / Industrial	Agriculture	Forest	Open Water
0301	384.0	148.1	584.6	480.1	136.4
0302	202.2	22.3	232.9	609.0	1.1
030201	113.7	0.0	93.5	165.8	0.0
0303	4.4	1.8	4.4	2.8	0.0
030301	157.5	37.1	114.7	490.4	0.0
0304	257.9	86.2	245.4	614.1	1.4
030401	78.3	9.2	214.7	355.0	0.6
0305	2.0	0.0	8.6	5.2	0.0
030501	104.6	8.1	357.3	1154.8	1.5
0306	322.1	5.8	512.0	958.5	1.5
030601	151.9	17.8	71.9	381.3	1.7
0307	236.5	9.6	391.3	328.0	3.3
030701	285.1	148.2	47.4	373.7	1.2
0308	176.9	1.0	281.9	590.4	0.7
030801	163.9	5.0	198.3	498.6	0.0
Total Acres by Major Land Use	2640.9	500.2	3358.9	7007.6	149.2

## **Channelization and Impoundments**

Of the 439,000 feet of stream that were mapped in the IPSI process, 31,374 feet of stream (7.1%) were identified as channelized. Channelized streams included no perennial streams, and most segments were associated with roads. (TVA, 2003)

The main channel of Stock Creek is conspicuously straight from the confluence with Casteel Branch extending upstream about 1.6 miles. The TVA/USGS 1:24,000 scale quadrangle map published in 1936 also shows this segment as straight, suggesting this segment of stream is either naturally straight or was straightened sometime before 1936.

There are no major impoundments in the watershed, but farm ponds are common. Stock Creek discharges to the Little River embayment of Fort Loudoun Reservoir, and the creek is impounded most of the year from its mouth to approximately mile 2.5. (TVA, 2003)

## **Sewer/Septic Systems**

The Stock Creek Watershed is impaired by elevated levels of bacteria, in part due to malfunctioning septic systems. Typically, the age of the system, lack of maintenance, an inadequate drain field, or poor soil quality can cause these problems.

Sewer service has been added in a piecemeal fashion, with no overall plan for comprehensive service. Future improvements may require a new pressure main and would benefit from Knox County and the utility company working together toward a coordinated plan.

## **Surface Water**

There are no community surface drinking water intakes in the watershed.

## **Groundwater**

Many houses are likely served by private wells. Unfortunately, the Stock Creek Watershed is underlain mainly by karstic carbonate rock which provides conditions favorable to rapid transport of pathogen-contaminated groundwater either to drinking water wells or back to surface water through seeps, springs, and fractures. (Gentry, 2006)

## **Streambank Erosion and Stream Buffers**

Of the 439,000 feet of stream that were mapped in the IPSI process, 44,918 feet of streambank (5.1%) were identified as actively eroding.

Buffers are generally not included in the management of agricultural and residential areas in the Stock Creek Watershed. Most creeks that are not in the woods have at most one row of trees in their buffer.

### 3.0 Causes and Sources of Pollution\*

TDEC has identified approximately 16 stream miles of impaired waters in the Stock Creek Watershed (Table 8). (TDEC, 2006a)

**Table 8 Stream Miles Identified by TDEC as Impaired**

Waterbody ID	Impacted Waterbody	County	Miles/Acres Impaired	CAUSE / TMDL Priority	Pollutant Source	COMMENTS
TN06010201066 – 0100	CASTEEL BRANCH	Knox	2.0	Loss of biological integrity due to siltation NA	Pasture Grazing Discharges from MS4 area	Stream is Category 4a. Impaired, but EPA has approved a siltation TMDL that addresses known pollutants.
TN06010201066 – 0200	TWIN BRANCH	Knox	1.87	Habitat loss due to alteration in stream-side or littoral vegetative cover Loss of biological integrity due to siltation NA NA	Pasture Grazing Discharges from MS4 area	Stream is Category 4a. Impaired, but EPA has approved siltation and habitat alteration TMDLs that address the known pollutants.
TN06010201066 – 0400	GRANDVIEW BRANCH	Knox	1.7	Escherichia coli NA	Discharges from MS4 area	Stream is Category 4a. EPA has approved a pathogen TMDL that addresses the known pollutant.
TN06010201066 – 0500	MCCALL BRANCH	Knox	1.73	Loss of biological integrity due to siltation NA	Discharges from MS4 area Streambank Modification	Stream is Category 4a. Impaired, but EPA has approved a siltation TMDL that addresses the known pollutant.
TN06010201066 – 0600	HIGH BLUFF BRANCH	Knox	1.25	Escherichia coli NA	Discharges from MS4 area	Stream is Category 4a. EPA has approved a pathogen TMDL that addresses the known pollutant.
TN06010201066 – 1000	STOCK CREEK	Knox	3.77	Physical Substrate Habitat Alterations Loss of biological integrity due to siltation Escherichia coli NA NA NA	Pasture Grazing Channelization	Stream is Category 4a. Impaired, but EPA has approved siltation, pathogen, and habitat alteration TMDLs that address known pollutants.
TN06010201066 – 1200	GUN HOLLOW BRANCH	Knox	1.36	Escherichia coli NA	Pasture Grazing	Stream is Category 4a. Impaired, but EPA has approved a pathogen TMDL that addresses the known pollutant.
TN06010201066 – 2000	STOCK CREEK	Knox	1.98	Escherichia coli NA	Pasture Grazing	Stream is Category 4a. Impaired, but EPA has approved a pathogen TMDL that addresses the known pollutant.

A more precise determination of the causes and sources of pollutants in the Stock Creek Watershed has been made based on modeling of watershed features and on water sample analyses.

#### Modeling of Watershed Features

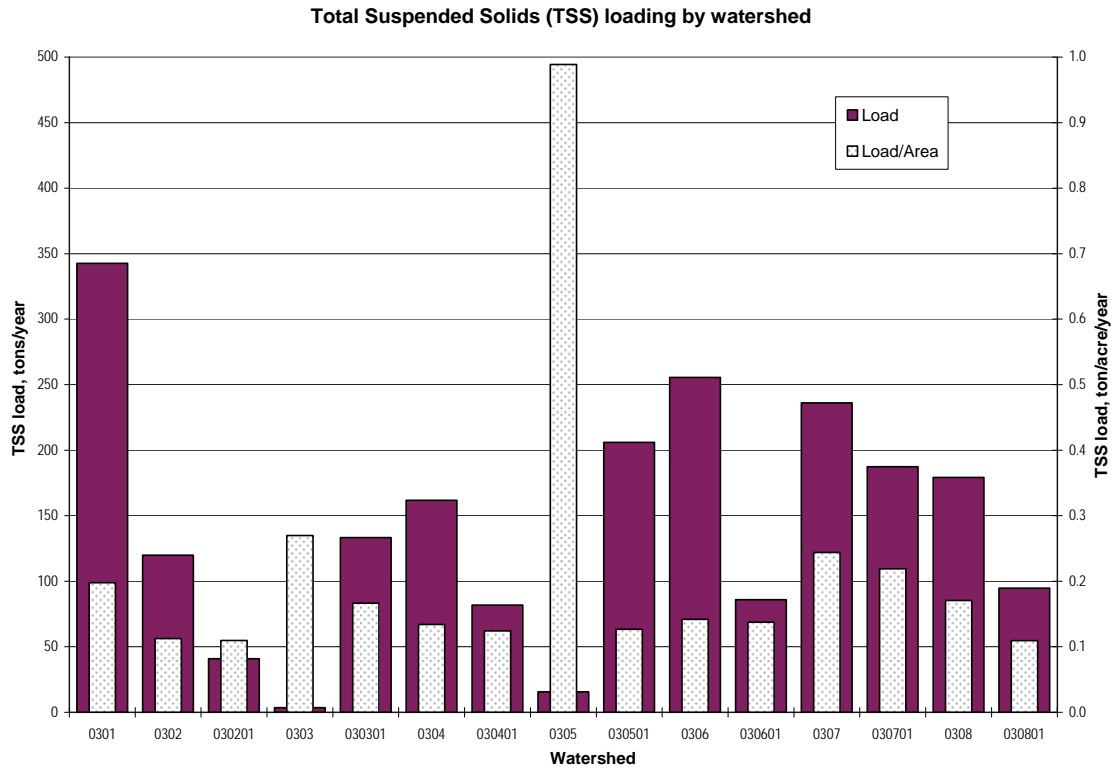
In addition to providing information on watershed features through aerial photography, the IPSI system can be used with other tools to locate known or suspected nonpoint sources of pollution. These tools include a nonpoint source (NPS) pollution inventory, an atlas that summarizes and displays NPS information, a desktop geographic information system (GIS) that allows access to the database, and a spreadsheet-based Pollution Loading Model (PLM) used to estimate pollutant loads by source and watershed (TVA, 2003). For an example of how the IPSI system was used to identify sources of pathogens in the Stock Creek watershed, see the paragraph entitled “Human Sources,” later in this section.

### **Agricultural Sources**

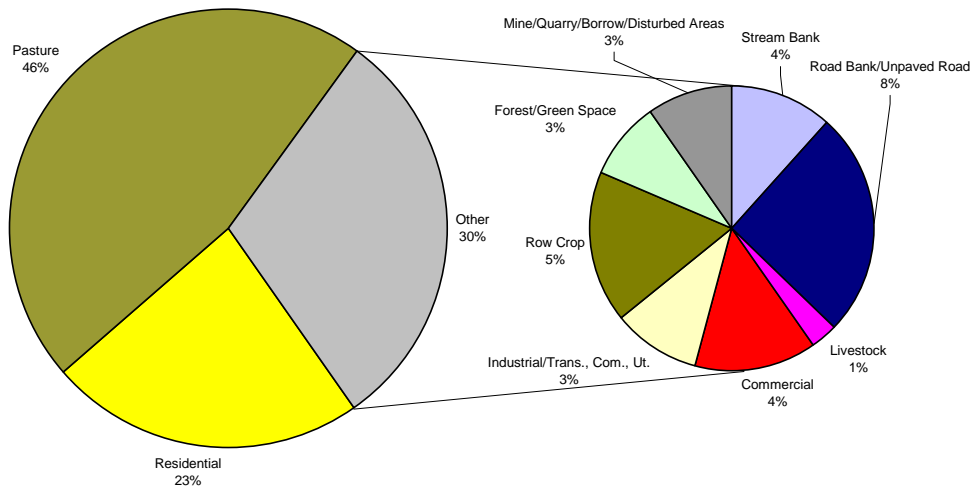
To help identify agricultural sources of sediment and bacteria and the impact of pasture quality on sediment and bacteria load, pasture conditions were classified in the IPSI database according to vigor and type of vegetation present at the time of the photography. Pastures were categorized in three types: good, fair, and overgrazed. Overgrazed pasture has the least ground coverage, often indicative of increased runoff of pollutants and increased erosion. Most of the pasture in the Stock Creek Watershed is classified as fair pasture (81%). The remainder of the pasture is classified as overgrazed (19%) or as good pasture (2%). (TVA, 2003.) See Appendix B for detailed data on the impact of pasture quality on sediment and bacteria load.

### **Human Sources**

To track down human sources of pathogens and sediment, annual *E. coli* and TSS loads were estimated in the PLM for each land use and subwatershed based on the NPS inventory. GIS coverage of sewered areas was obtained from Knox-Chapman Utility District, and this coverage was combined with the IPSI data to determine the area of unsewered residential area in each subwatershed. It was then assumed that the average lot size is one acre, 10% of systems are failing (a high rate that reflects the poor soil conditions for septic systems in the watershed), and 5% of the  $150 \times 10^{12}$  colony forming units (CFU) per year generated by the average household are delivered to the stream. A higher failure rate (30%) was assumed in the High Bluff area (subshed #0307) to account for the higher bacteria loads measured from this area. Results of this analysis are shown in Figures 8 through 11.

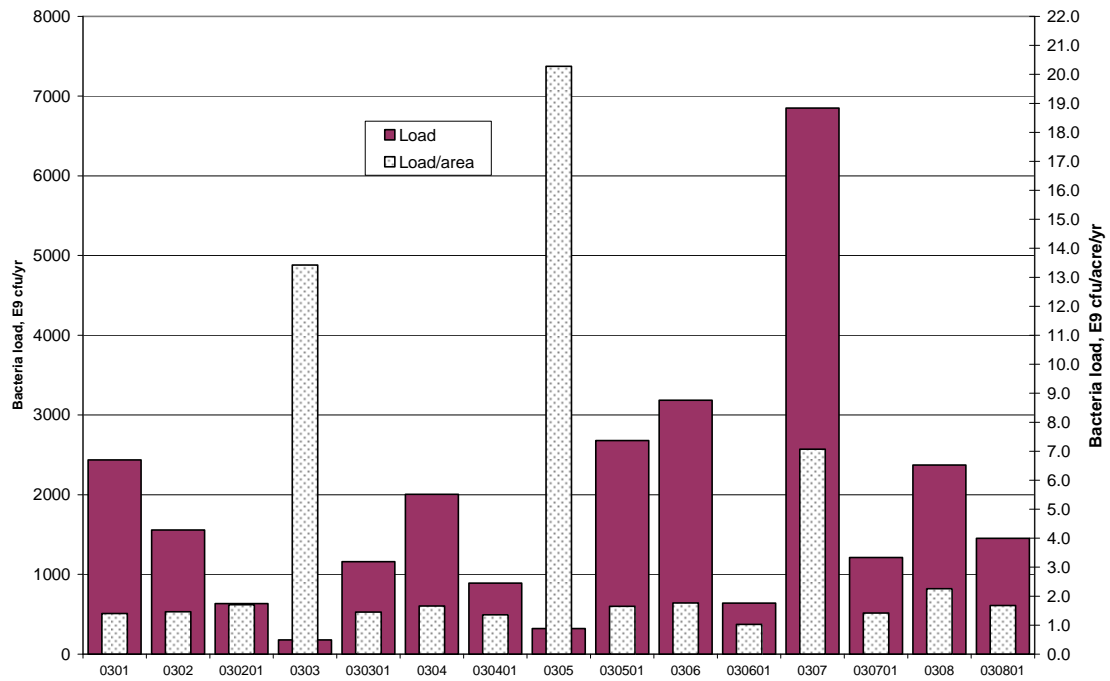


**Figure 8 Total Suspended Solids (TSS) Loading by Subwatershed**

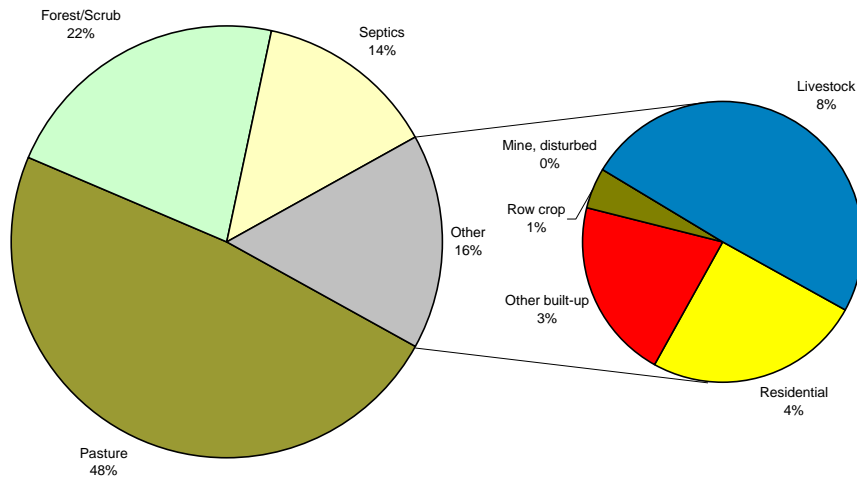


**Figure 9 Total Suspended Solids (TSS) Load by Source**





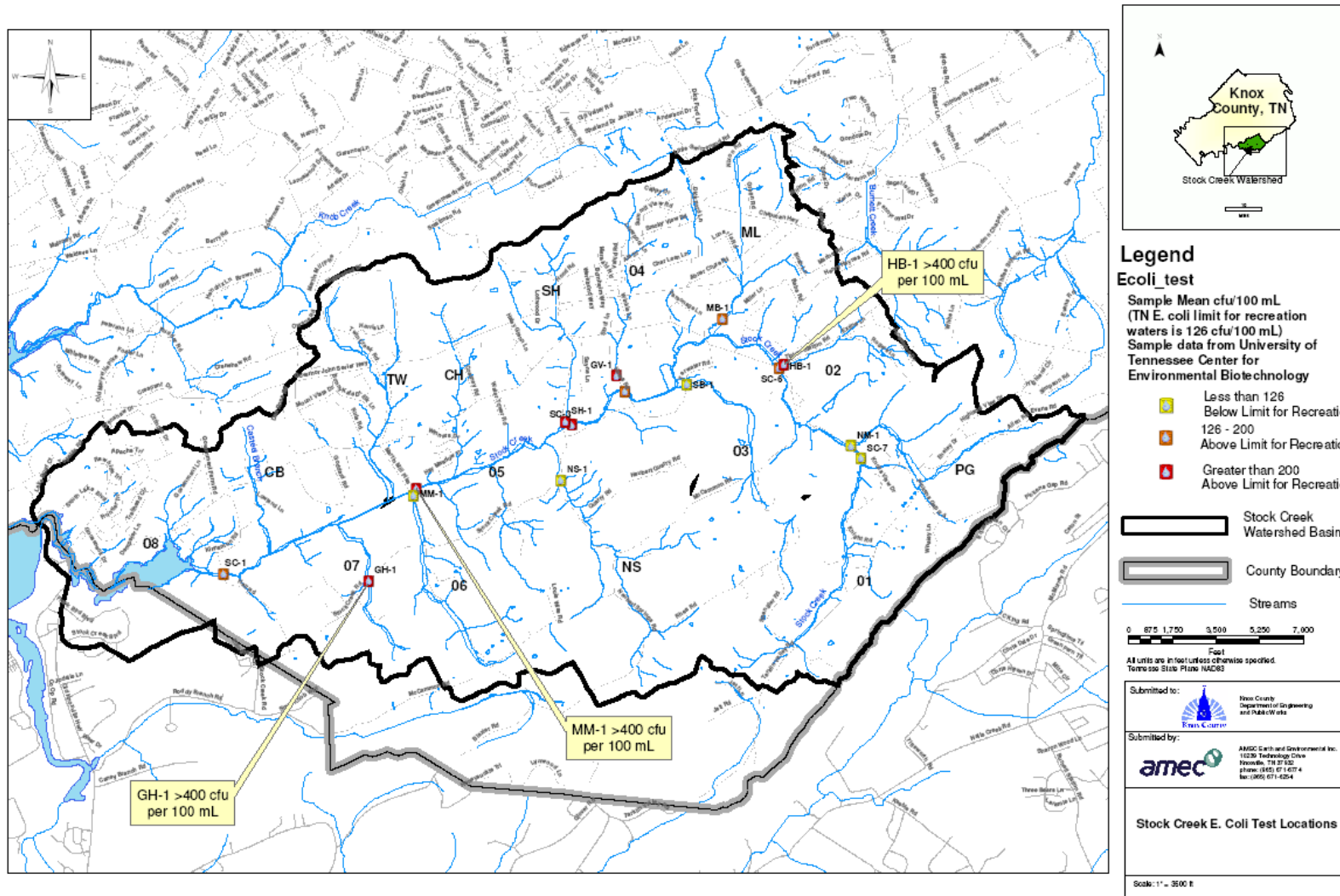
**Figure 10 *E. coli* Load by Subwatershed**



**Figure 11 *E. coli* Load by Source**

### Monitoring

Sixteen sites were selected for pathogen sampling in the Stock Creek Watershed (Figure 12). Samples were collected and analyzed for bacterial pathogens, with duplicate samples being taken and analyzed using an innovative technique involving real-time PCR analysis and DNA sequence analysis. This technique allows pathogen sources to be differentiated as to whether the source is human, cattle, horse, or other, so that restoration strategies can be better tailored to particular sources in particular subwatersheds. For details of the sampling and analysis techniques, as well as the resulting data, see Appendix C.



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Figure 12 *E. coli* Monitoring Sites

Initial monitoring revealed high levels of pathogens attributable to bovine fecal contamination at several locations in the Stock Creek Watershed (Table 9). However, several locations were identified that had high levels of fecal contamination not attributable to cattle. Further studies were conducted to identify the origin of fecal contamination at these sites (human versus horses versus wildlife). Sites were examined at high, medium and low water flows to determine whether the predominant source of fecal contamination differed with rates of flow in the watershed.

**Table 9 Pathogen Assessment of Sub-Watersheds in the Stock Creek Watershed**

<b>Sub-Watershed</b>	<b>Sampling Site (s)</b>	<b>Pathogen Assessment* (The <i>E. coli</i> recreational limit is 126 CFU/100ml)</b>
01	SC-7	66% of samples <b>below</b> recreational limit (Geomean=126), 40% attributable to cattle
PG	NM-1	75% of samples <b>below</b> recreational limit (Geomean=111), 15% attributable to cattle
02	SC-6, HB-1	83% of samples <b>above</b> recreational limit (Geomean= 313), 33% attributable to cattle 100% of samples <b>above</b> recreational limit (Geomean= 414), 4% attributable cattle
ML	SC-5 MB-1	83% of samples <b>above</b> recreational limit (Geomean=516), 27% attributable to cattle 63% of samples <b>above</b> recreational limit (Geomean=208), 6% attributable cattle
03	SB-1	75% of samples <b>below</b> recreational limit (Geomean= 68) , 3% attributable cattle
04	SC-4 GV-1	92% of samples <b>above</b> recreational limit (Geomean=462), 21% attributable to cattle 83% of samples <b>above</b> recreational limit (Geomean=346), 1% attributable to cattle
NS	NS-1	75% of samples <b>above</b> recreational limit (Geomean=224), 41% attributable to cattle
05	No sites	Not tested
CH	No sites	Not tested
06	SC-2 MM-1	92% of samples <b>above</b> recreational limit (Geomean=348), 29% attributable to cattle 83% of samples <b>above</b> recreational limit (Geomean=251), 18% attributable to cattle
TW	No sites	Not tested
07	GH-1	100% of samples <b>above</b> recreational limit (Geomean= 455), 84% attributable to cattle
CB	No Sites	Not tested
08	SC-1	75% of samples <b>above</b> recreational limit (Geomean 245), 31% attributable to cattle

\* Geomeans in this study do not represent regulatory geomeans.

Across the watershed, sequences identified with high confidence were predominantly assigned to either humans (63%) or cattle (33%). Another 4% of the sequences were attributed to horses or other animals.

When considered on a site-to-site basis, 12 out of 13 sites (92%) had sequences of human fecal origin (14% to 63% of all sequences). Six sites contained both sequences of human and cattle fecal origin. Only one site contained only sequences of cattle origin (GH-1), whereas four sites only contained sequences of human origin (NS-1, GV-1, MB-1, HB-1). (Layton, 2006)

### **Point vs. nonpoint sources**

There are no point sources in the Stock Creek Watershed.

## 4.0 Estimated Load Reductions\*

Total existing TSS load is estimated by PLM modeling to be 2145 tons/year. Total *E. coli* loading is estimated as  $27,575 \times 10^9$  colony forming units (CFU) per year. See Section 3.0 for details about modeling procedures and results.

Numeric targets are based on the final TMDL for the watershed (see Section 1.0). The TMDL requires a load reduction of 88% for *E. coli* and 35.3% for sedimentation. For the first five years of funding (Phase 1), this plan is designed to accomplish one-third of this goal, or a 29% reduction in *E. coli* loading and a 12.9% reduction in sedimentation.

## **5.0 Restoration Strategies and Best Management Practices\***

### **Subwatershed Strategy**

The Stock Creek Watershed has been subdivided into 15 smaller drainage basins, or subwatersheds, based on drainage patterns. Water quality monitoring, combined with targeted analysis, has provided the ability to determine which areas are the primary contributors and whether the sources are human or bovine. Where the source is bovine, agricultural BMPs will be implemented to reduce pathogen levels. Where the source is human, conditions such as soil quality, sewer/septic availability, and available suitable space have been studied to determine whether expanded sewer service might be the most appropriate solution for a particular area. Where modeling shows there are not a lot of failing septic systems, then a closer look is taken at sewer lines.

### **Agricultural Sources**

Conservation plans will be developed for each participating farm. Recommended systems of BMPs will vary for each farm and will include the following practices:

- Pasture and Hayland Planting - Establishing native or introduced forage species
- Prescribed Grazing - Managing the controlled harvest of vegetation with grazing animals
- Fence - A constructed barrier to animals or people
- Filter Strip - A strip or area of herbaceous vegetation between cropland, grazing land, and environmentally sensitive areas
- Riparian Forest Buffer - An area of trees and/or shrubs located adjacent to and up-gradient from water bodies
- Pipeline - Pipeline having an outside diameter of eight inches or less to convey water for humans or livestock
- Watering Facility - A device for providing animal access to water
- Heavy Use Area Protection - The stabilization of areas intensively used by people, animals, or vehicles by surfacing with a suitable material
- Streambank and Shoreline Protection - Treatment used to stabilize and protect banks of streams and other bodies of water
- Stream Channel Stabilization - Stabilizing the channel of a stream with suitable structures

### **Miscellaneous BMPs**

Other Best Management Practices that will be employed are:

- Spring Development - Improving springs and seeps by excavating, cleaning, capping, or providing collection and storage facilities
- Stream Crossing - A travelway constructed across a stream to allow livestock, people, and equipment to cross with minimal disturbance

- Grade Stabilization Structure - A structure used to control the grade and head cutting in natural or artificial channels
- Grassed Waterway - A natural or constructed channel that is shaped to required dimensions and established with suitable vegetation
- Critical Area Planting - Planting vegetation such as trees, shrubs, grasses, and legumes on highly or critically eroding areas
- Stream Habitat Enhancement Projects

A package of practices was developed from the above list that is representative of conservation plans for the Stock Creek watershed. This package (Table 10) was used for estimating load reductions and treatment costs on a per-acre basis. For estimating load reductions, this set of practices was applied in the PLM to determine the extent of treatment required to meet loading goals. Once this was determined, costs could be readily estimated.

In order to reach Phase I bacteria loading goals, the model indicates that 1100 acres of overgrazed and fair pasture require treatment (34% of total pasture). As part of the pasture conservation package, 2600 feet of stream bank will be stabilized, 47,000 feet of stream will be buffered, and about 470 head of cattle will be excluded from streams. The total estimated cost would be \$822,000. At a cost share rate of 80%, \$657,000 will be provided by grants or other sources. Treatment adequate to reduce bacteria loading to target levels will also reduce sediment loading enough to meet the target.

**Table 10 Per Acre BMP Costs**

<b>Units Treated Per Acre</b>	<b>Unit</b>	<b>Cost Per Unit</b>	<b>Total Cost per acre for described treatment</b>
1	Acre Pasture Renovation	\$150.00	\$150.00
50	Ft cross fence	\$2.50	\$125.00
0.01	Water and HUAP	\$20,000.00	\$200.00
0.017	Acre Buffer (based on 20 ft width and 37 ft in length)	\$6,000.00	\$102.00
2.3	Ft stream bank stabilization	\$45.00	\$105.14
	Misc -- critical area, stream crossings		\$60.00
<b>Total Cost of Pasture Package per acre</b>			<b>\$742.14</b>



## **Strategies for Human Sources**

Human sources are caused by failing septic systems and in some cases, straight piping of sewage into the stream. Treatment costs were developed by subwatershed, based on the type of treatment required in a particular area, and recognizing the limitations imposed by soil and other physical characteristics. In certain cases, where sewer connections are not available and septic repair is not an option, then alternative solutions may be necessary such as distributive systems. Average cost per household for the Stock Creek Watershed was estimated to be \$11,420.

According to model results (Section 3.0), 86 systems, or 33% of failing systems in the watershed, must be treated to reach Phase 1 goals. Cost of this improvement was estimated at \$988,000.

## **New County Stormwater Ordinance**

In 2006, Knox County's stormwater ordinance was updated with recommendations from the Knox County Site Planning Roundtable. Community leaders with diverse perspectives on development and environmental protection achieved consensus on how to enhance the ordinance to address non-structural control options, such as low impact development (LID), stream buffers, open space, and conservation easements. The updates also will enhance water quality-based design standards for both structural and non-structural options. The resulting ordinance is directly targeted at implementing priority recommendations of the 2003/2005 Assessment, which include but are not limited to:

- Flood Mitigation—e.g., determining best use of undeveloped parcels, bond-funded
- Environmental restoration, encouraging/requiring good landscape design
- Wetlands Preservation and Mitigation—e.g., easements, acquisitions, and restoration
- Streambank Stabilization—e.g., bank restoration and riparian buffers with native plants
- Slope and Ridgetop Protection—e.g., limits on development, land use activities, easements
- Parks and Greenways—easements, land acquisition, greenway enhancement, new parks

## 6.0 Information and Education\*

The information/education component has been designed to enhance public understanding of the project and encourage early and continued community involvement. Three years ago, Stock Creek Watershed Initiative partners developed an outreach/education plan that includes goals and objectives, key messages, planned and actual activity completion dates, and measures for identifying success. The plan has been revised and updated each year and is designed to get key messages to our target audiences while keeping us focused.

A three-tiered approach has been taken in order to reach target audiences with key messages and provide them with opportunities for involvement. First, the focus is on building awareness, filling in knowledge gaps, and clearing up misconceptions. Second, more extensive education through workshops, brochures, etc. takes place. Third, specific ways are identified to involve each of the audience members so they gain a sense of ownership of the watershed and put into practice the key messages.

Target audiences in the Stock Creek Watershed include farmers, rural and suburban residents, local organizations and businesses, local developers, builders, subcontractors, and utilities. Primary messages that have been identified as currently important to convey include:

- A watershed is an area of land that drains to a waterbody. The Stock Creek Watershed drains approximately 21 square miles.
- Activities throughout the watershed can have a substantial impact on its water quality.
- Failing septic systems, poor agricultural practices, and suburban development in the Stock Creek Watershed are impacting creek water quality with increased bacteria concentrations, sediment input, riparian habitat destruction, and cumulative input of household and business-generated pollutants.
- Each person plays a part in contributing to local water quality problems and each of us can be a part of the solution.
- Here are ways to make a difference .... here is how to become involved...

The Initiative partners have invested five years in improving the water quality in the Stock Creek Watershed including initiating a comprehensive approach to building community awareness about local watershed issues and educating and involving targeted audiences in watershed involvement projects. However, with continued residential and commercial growth in the Stock Creek Watershed, the large number of septic system and agriculture problems, and its continued listing on the TDEC 303(d) list, there is much yet to be done. The following list shows past/current, and future education and outreach strategies.

Awareness strategies:

Past/Current

- Maintaining a presence in the media
- Conducting civic and community presentations

- Creating stormwater management technique demonstration sites
- Updating website

Future

- Posting watershed entry signs
- Bi-annual newsletter
- Yearly calendars

Educational strategies:

Past/Current

- Kids-in-the-Creek
- Adopt-A-Watershed
- Targeted community meetings

Future

- Construction site stormwater management program
- Farmer's breakfast meetings
- Bonny Cate Festival

Involvement strategies:

Past/Current

- Adopt-A-Stream
- Adopt-A-Watershed service projects
- Community-wide creek clean-ups

Future

- Riparian restoration with native seedling give-away
- Bi-annual residential NPS workshops
- Environmental Stewardship Program (ESP)

In addition, SCWI partners accomplished the following educational tasks:

- Developed, published and distributed Stock Creek Watershed brochure.
- Developed, published and distributed Septic System Maintenance brochure.
- Partnered with the TN Water Resources Research Center to implement the Adopt-A-Watershed Program in South Doyle High School and Middle School
- Gave educational presentations to stakeholder groups.

Initiative partners plan to maintain and/or expand the scope of its existing projects while adding new projects designed to deepen the knowledge and involvement of watershed residents. Initial plans for new project strategies are listed in Section 7.0, although all strategies will be periodically re-evaluated and adapted as necessary to ensure their relevance and effectiveness.

## 7.0 Implementation Plan\* and Milestones\*

Calendar year	2007				2008				2009				2010				2011				2012			
Calendar quarter	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4			
<b>Activity</b>																								
<b>INFORMATION AND EDUCATION</b>																								
<b>General Education</b>																								
Publish newspaper articles	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Publish bi-annual newsletter			x		x			x				x				x			x			x		
Manage website		x		x		x		x		x		x		x		x		x		x		x		
Kids-in-the-Creek			x					x					x					x				x		
Implement Adopt-A-Watershed in 2 schools	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Develop yearly calendar			x					x					x					x				x		
<b>Ag Education</b>																								
Conduct quarterly farmer's breakfast meetings	x	x	x	x	x	x	x	x	x				x				x					x		
Farm Tours									x				x									x		
Restore riparian habitat			x					x					x									x		
<b>Suburban/Urban Education</b>																								
Conduct bi-annual NPS workshops			x	x				x	x				x	x				x	x			x		
Develop sewer/septic protocol with Knox Co. Health Dept	x																							
Develop sewer connect education materials				x	x																			
<b>Outreach Activities</b>																								
Conduct sewer connect community fairs								x	x															
Implement Adopt-a-Stream		x						x					x									x		
Host creek clean up event (CPR)	x				x					x				x								x		
<b>AG &amp; STORMWATER BMPs</b>																								
Ag BMPs	x		x	x	x			x	x	x			x	x	x			x	x	x		x		
Septic Repairs		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Sewer extensions				x	x			x	x	x			x											
Sewer connections								x	x	x			x	x	x			x	x	x				
<b>MONITORING</b>																								
Monthly physical, chemical, bacteriological	x	x	x	x														x	x	x	x			
Quarterly monitoring physical, chem, bact								x	x	x	x	x	x	x	x	x								
Collect and analyze rain event samples	x	x	x	x																				
Develop rating curves	x	x	x	x	x	x	x	x	x	x	x	x	x											
Flow with Staff Gages	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Benthic community samples at 5 sites	x																					x		
Habitat assessment at 9 sites	x																					x		
Fish community assessment at 1 site	x																					x		
<b>EVALUATION</b>																								
Compile and analyze quarterly monitoring results					x				x				x					x				x		
Evaluate progress, adapt monitoring plan, if necessary					x				x				x					x				x		
Evaluate+A47 success in achieving reduction goals																						x		
Adapt Watershed Action Plan as needed																						x		

## 8.0 Monitoring\* and Evaluation\*

Physical, chemical, bacteriological and biological conditions will be monitored to document baseline conditions, refine pollution source identification, track progress and evaluate the success of efforts to restore the streams of Stock Creek Watershed and remove them from the 303(d) list. All monitoring will follow TDEC Standard Operating Procedures. The monitoring plan is outlined below.

### Pathogens

The TMDL for Pathogens for the Little River Subwatershed (TDEC, 2005) calls for an 88.0% reduction in *E. coli* loads in four HUC-12 subwatersheds: Grandview Branch, High Bluff Branch, Stock Creek and Gun Hollow Branch. In order to track progress towards achieving water quality goals, *E. coli* instream grab samples will be collected at nine sites monthly during year one and year four and quarterly during year two and three of this initiative. Five of the nine sites are established TDEC monitoring locations. The additional four sites were chosen to establish baseline conditions in subwatersheds that are targeted for restoration activities. In addition to the base flow samples, four high flow sampling events will be collected each year. TDEC defines a high flow event during a wet season (January through March) as an event with greater than 0.25 inches of rain within the last 24 hours prior to sample collection. Dry season (August through October) high flow events are defined as precipitation greater than 0.50 inches of rain within the last 24 hours prior to sample collection.

Results will be compared with State standards to evaluate the success of this initiative. The goal is to document that Stock Creek and its tributaries meet State bacteriologic standards by 2012, and to initiate a process to remove the streams of Stock Creek watershed from the 303(d) list.

### Siltation and Habitat Alteration

Numeric water quality criteria have not been established for siltation and habitat alteration impairments in Tennessee. The TMDL for Siltation and Habitat Alteration for Ft. Loudoun Lake Watershed was based on a numeric interpretation of the narrative water quality standard for protection of fish and aquatic life. Average annual sediment loading was derived from biologically healthy watersheds, located within the same ecoregion. The geometric mean of average annual sediment loads of the reference watersheds serve as target values for the Siltation and Habitat Alteration for Ft. Loudoun Lake Watershed TMDL. The TMDL calls for a 35.3% reduction in annual sediment load in Casteel Branch, Twin Branch, McCall Branch and Stock Creek.

Our strategy for evaluating the success on this initiative in reducing sediment load and removing Stock Creek and the impaired tributaries from the 303(d) list will be to document that benthic macroinvertebrate and physical habitat scores meet State standards. Benthic community (square kick protocol) and physical habitat will be assessed at five sample sites established by TDEC during year one and year four of this initiative.

In addition to benthic community and physical habitat assessments, the following monitoring will be performed to better identify sediment sources and track interim progress:

- Total suspended solids (TSS) samples will be collected using depth integrated samplers. Sample sites will be the same nine sites sampled in for *E. coli*. Base flow samples will be collected monthly during year one and year four and quarterly during years two and three, coinciding with the *E. coli* monitoring. At least four high flow event samples will be collected each year, as defined in the previous section. Results will be compared with concentrations in ecoregion reference watersheds and year one baseline data. The goal is for median base flow TSS concentrations at each site to be less than or equal to 5 mg/L (90<sup>th</sup> percentile of TSS data from ecoregion reference watersheds).
- Single stage samplers (containers triggered to collect automatic samples for high flow in streams) will be installed at five sites along Stock Creek and the sediment impaired tributaries to identify sources of increased TSS concentrations within these reaches and document the sediment load contributed to the streams from high flow events. TSS samples will be collected and analyzed after each significant storm.

## **Flow**

Staff gages will be placed at the nine sample sites. (Staff gages are used for measuring water levels in lakes, rivers, reservoirs, and other bodies of surface water; these gages provide a visual indication of water level, and are designed for high accuracy and excellent readability). All gages are accurately graduated and will be fastened to walls, piers, and other structures in the stream. Rating curves will be developed and used to estimate flows. The gages will be routinely maintained by monitoring staff, and replaced as needed.

## **Additional Assessments**

During grab sampling, multiparameter probes will be used to assess dissolved oxygen, conductivity, temperature and pH.

Habitat assessments will be performed within the immediate vicinity of the nine sample sites. This will aid in the interpretation of sediment loading sources.

Stock Creek's fish assemblage has been assessed several times at Mile 4.4 since 1996, using the Index of Biotic Integrity (IBI). Scores vary from 24 (very poor/poor) in 1996 to a rating of 38 (poor/fair) in 2001. Most IBI scores for the last decade have been poor. The most recent IBI scores (from 2006) show a rating of 34 (poor). (TVA, 2006)

According to data reported by the Water Quality Forum from Mile 5 between 2002 and 2004, 11-12 species of native fish were typically identified. (WQF, 2004)

## **Evaluation and Adaptive Management**

This watershed action plan outlines strategies to be implemented over a five-year period. The goal is restore the water quality of Stock Creek and its tributaries by 2012. Sampling results will be reviewed annually to ensure that progress is being made. If necessary, restoration priorities and strategies will be reevaluated and adapted. Future plans will be developed and evaluated on a five-year cycle that would be closely coordinated with TDEC's watershed management cycle.

As part of the adaptive management strategy, additional study of channelization and its impacts to siltation and habitat alteration may be performed in future years. Studies to further identify and quantify the effects of channel alteration could provide useful information in determining if management strategies such as reintroducing sinuosity, reconnecting floodplains or adding instream structures to dissipate energy and increase habitat are more suitable approaches for stream restoration than more traditional Best Management Practices. Channelization studies, visual streambank assessment, erosion pins, benthic communities and pebble counts, could include comparative assessments of channelized and nonchannelized reaches.

## 9.0 Estimated Budget and Sources of Funding\*

**Table 11 Budget for Phase I of the Restoration Plan**

Budget category	319(h) funding	Grantee match		Non-Matching contributions		Total
		Funds	Funding source	Funds	Funding source	
<b>Outreach and Education</b>						
Salary and benefits		\$45,500	SCWI partners			\$45,500
Printing, rentals	\$10,000	\$11,000	Knox Co., TVA			\$21,000
Supplies	\$25,000					\$25,000
Programming		\$50,000	SCWI partners, land owners			\$50,000
<b>BMPs/retrofits</b>						
AG - implementation	\$357,000	\$165,000	SCWI partners, land owners	\$300,000	NRCS programs	\$822,000
Urban - implementation	\$300,000	\$100,000	SCWI partners, land owners	\$588,000	Knox County	\$988,000
Technical assistance	\$50,000			\$150,000	NRCS	\$200,000
Salary and benefits		\$100,000	SCWI partners			\$100,000
<b>Monitoring</b>						
Salary and benefits		\$10,000	SCWI partners			\$10,000
Lab analysis		\$77,500	TDEC			\$77,500
<b>Evaluation</b>						
Salary and benefits		\$10,000	SCWI partners			\$10,000
<b>Project Management</b>						
Reports		\$25,000	Water Quality Forum			\$25,000
<b>Total</b>	\$742,000	\$594,000		\$1,038,000		\$2,373,500



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# Appendix A

## Description of Federal and State Ranks & Status Codes

**GLOBAL RANK** - The global or world-wide rank of a species which is a non-legal rank indicating the rarity and vulnerability of a species

<b>G1</b>	Extremely rare and critically imperiled in the world with five or fewer occurrences, or very few remaining individuals, or because of some special condition where the species is particularly vulnerable to extinction
<b>G2</b>	Very rare and imperiled within the world, six to twenty occurrences, or few remaining individuals, or because of some factor(s) making it vulnerable to extinction
<b>G3</b>	Rare and uncommon in its range or found locally in a restricted range, generally from 21-100 occurrences
<b>G4</b>	Widespread, abundant, and apparently secure globally, but with cause for long-term concern
<b>G5</b>	Demonstrably widespread and secure globally
<b>GH</b>	Of historical occurrence throughout its range, e.g. formally part of the established biota, with the expectation that it may be rediscovered
<b>GU</b>	Can not be ranked using available information
<b>GX</b>	Believed to be extirpated throughout its range
<b>HYB</b>	Hybrid within its range in Tennessee
<b>SSYN</b>	Synonym for another species
<b>_Q</b>	Questionable taxonomy (GRANKs only)
<b>_T#</b>	Subspecific taxon rank (GRANKs only)

**STATE RANK** - The state rank of a species in Tennessee. Like the G\_rank this is a non-legal rank indicating the rarity and vulnerability of a species at the state level.

<b>S1</b>	Extremely rare and critically imperiled in the state with five or fewer occurrences, or very few remaining individuals, or because of some special condition where the species is particularly vulnerable to extinction
<b>S2</b>	Very rare and imperiled within the state, six to twenty occurrences, or few remaining individuals, or because of some factor(s) making it vulnerable to extinction
<b>S3</b>	Rare and uncommon in the state, from 21-100 occurrences
<b>S4</b>	Widespread, abundant, and apparently secure within the state, but with cause for long-term concern
<b>S5</b>	Demonstrably widespread and secure in the state
<b>SH</b>	Of historical occurrence in Tennessee, e.g. formally part of the established biota, with the expectation that it may be rediscovered
<b>SU</b>	Can not be ranked using available information
<b>SX</b>	Believed to be extirpated from the state
<b>S#S#</b>	Denotes a "range rank" because the rarity of the species is uncertain (e.g. S1S3)
<b>S?, S_?</b>	Unranked at this time or rank uncertain
<b>SE</b>	Exotic species established in the state
<b>SE#</b>	Exotic numeric (e.g. the Asian clam <i>Corbicula fluminea</i> would be SE5)
<b>SP</b>	Potentially occurring in Tennessee, but not yet documented by DNH
<b>_N</b>	Occurs in Tennessee in a non-breeding status (mostly applies to vertebrates)

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## Description of Federal and State Ranks & Status Codes

<b>_B</b>	Breeds in Tennessee
<b>SA</b>	Accidental or casual in the state (several birds)
<b>SR</b>	Reported from the state, but insufficient data to assign rank
<b>SRF</b>	Reported falsely from the state
<b>HYB</b>	Hybrid within its range in Tennessee
<b>SSYN</b>	Synonym for another species
<b>_Q</b>	Questionable taxonomy (GRANKs only)
<b>_T#</b>	Subspecific taxon rank (GRANKs only)

### FEDERAL STATUS - The federal listing under the U.S. Endangered Species Act

<b>LE, Listed Endangered</b>	Taxon is threatened by extinction throughout all or a significant portion of its range
<b>E/SA, Endangered by Similarity of Appearance</b>	Taxon is treated as an endangered species because it may not be easily distinguished from a listed species
<b>LT, Listed Threatened</b>	Taxon is likely to become an endangered species in the foreseeable future
<b>T/SA, Threatened by Similarity of Appearance</b>	Taxon is treated as a threatened species because it may not be easily distinguished from a listed species
<b>PE, Proposed Endangered</b>	Taxon proposed for listing as endangered
<b>PT, Proposed Threatened</b>	Taxon proposed for listing as threatened
<b>C, Candidate species***</b>	Taxon for which the USFWS has sufficient information to support proposals to list the species as threatened or endangered, and for which the Service anticipates a listing proposal
<b>(PS) Partial Status (based on taxonomy)</b>	Taxon which is listed in part of its range, but for which Tennessee <u>subspecies</u> are not included in the Federal designation
<b>(PS:status) Partial Status (based on political boundaries)</b>	Taxon which is listed in part of its range, but for which Tennessee <u>populations</u> are not included in the Federal designation e. g. (PS:LE)
<b>(XN) Non-essential experimental population in portion of range</b>	Taxon which has been introduced or re-introduced in an area from which it has been extirpated, and for which certain provisions of the Act may not apply

## Description of Federal and State Ranks & Status Codes

STATE STATUS -The legal listing in Tennessee

<b>E, Endangered</b>	Any species or subspecies whose prospects of survival or recruitment within the state are in jeopardy or are likely to become so within the foreseeable future
<b>T, Threatened</b>	Any species or subspecies that is likely to become an endangered species within the foreseeable future
<b>D, Deemed in Need of Management</b>	Any species or subspecies of nongame wildlife which the executive director of the TWRA believes should be investigated in order to develop information relating to populations, distribution, habitat needs, limiting factors, and other biological and ecological data to determine management measures necessary for their continued ability to sustain themselves successfully. This category is analogous to "Special Concern."
<b>S, Special Concern</b>	Any species or subspecies of plant that is uncommon in Tennessee, or has unique or highly specific habitat requirements or scientific value and therefore requires careful monitoring of its status.

### *Additional Modifiers for Plants*

<b>PE, Proposed Endangered</b>	Any species or subspecies of plant nominated by the Scientific Advisory Committee to be added to the list of Tennessee's endangered species. After approval by the commissioner of the Dept. of Environment & Conservation and the concurrence of the commissioner of Agriculture, these plants will formally become State endangered.
<b>PT, Proposed Threatened</b>	Any species or subspecies of a plant nominated by the Scientific Advisory Committee to be added to the list of Tennessee threatened species. After a public hearing, these plants will formally become State threatened.
<b>E-PT, Endangered-Proposed Threatened</b>	Species which are currently on the state list of endangered plants, but are proposed by the Scientific Advisory Committee to be down-listed to threatened. After approval by the commissioner of the Dept. of Environment & Conservation and the concurrence of the commissioner of Agriculture, these plants will formally become State threatened.
<b>E-PS, Endangered Proposed Special Concern</b>	Species which are currently on the state list of endangered plants, but are proposed by the Scientific Advisory Committee to be down-listed to special concern. After approval by the commissioner of the Dept. of Environment & Conservation and the concurrence of the commissioner of Agriculture, these plants will formally become State special concern.
<b>T-PE, Threatened Proposed Endangered</b>	Species which are currently on the state list of threatened plants, but are proposed by the Scientific Advisory Committee to be listed on the state endangered list. After approval by the commissioner of the Dept. of Environment & Conservation and the concurrence of the commissioner of Agriculture, these plants will formally become State endangered.

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## Description of Federal and State Ranks & Status Codes

<b>T-PS, Threatened Proposed Special Concern</b>	Species which are currently on the state list of threatened plants, but are proposed by the Scientific Advisory Committee to be down-listed to special concern. After a public hearing, these plants will formally become State special concern.
<b>P, Possibly Extirpated</b>	Species or subspecies that have not been seen in Tennessee for the past 20 years. May no longer occur in Tennessee.
<b>C, Commercially Exploited</b>	Due to large numbers being taken from the wild and propagation or cultivation insufficient to meet market demand. These plants are of long-term conservation concern, but the Division of Natural Heritage does not recommend they be included in the normal environmental review process.

# Appendix B

**Table 12 Impacts from Pasture Quality**

Water-shed ID	Area (acres)			Sediment load (tons TSS/year)			Bacteria load (E9 cfu/year)		
	Good pasture	Fair pasture	Overgrazed Pasture	Good pasture	Fair pasture	Overgrazed Pasture	Good pasture	Fair pasture	Overgrazed Pasture
0301	1.5	484.1	47.0	67.8	110108.4	107019	0.35	584.5	590.9
0302		200.1	32.8	0.0	47136.1	77148	0.00	320.5	499.3
030201		93.4		0.0	26741.2	0	0.00	214.7	0.0
0303		4.4		0.0	2210.3	0	0.00	10.1	0.0
030301		110.8	3.9	0.0	30397.7	10588	0.00	175.9	58.5
0304		199.5	45.9	0.0	45803.1	105391	0.00	337.0	723.5
030401		183.3	31.4	0.0	43882.9	75235	0.00	116.2	260.4
0305		2.9	5.6	0.0	1460.4	28040	0.00	6.7	108.2
030501	3.5	230.8	110.9	134.4	44131.7	212076	0.72	251.6	1298.6
0306		404.1	106.6	0.0	81877.2	215971	0.00	378.4	1127.8
030601	1.9	67.0	3.0	105.2	18446.1	8152	0.81	125.4	49.8
0307		254.3	137.1	0.0	58903.5	317537	0.00	573.2	2597.8
030701		36.0	11.4	0.0	9111.3	28747	0.00	74.5	203.7
0308		202.6	62.6	0.0	44419.5	137212	0.00	247.9	793.2
030801		172.4	25.9	0.0	38489.9	57936	0.00	158.2	270.0
<b>Total</b>	<b>6.9</b>	<b>2645.8</b>	<b>624.1</b>	<b>307.3</b>	<b>603119.2</b>	<b>1381053</b>	<b>1.9</b>	<b>3574.8</b>	<b>8581.7</b>
<b>Percent of pasture total</b>	<b>0.2%</b>	<b>80.7%</b>	<b>19.0%</b>	<b>0.02%</b>	<b>30.4%</b>	<b>69.6%</b>	<b>0.02%</b>	<b>29.4%</b>	<b>70.6%</b>

# Appendix C

## Stock Creek Report: Summary of All Data Interpretations 2002-2005 (Alice Layton, 2006)

### Summary

Stock Creek is a relatively small watershed (20 square miles). The land use patterns consist of rural and small suburban subdivisions and small cattle grazing operations. A water quality assessment was performed between April 2003 and February 2004 with the goal of determining the level of pathogen contamination in the Stock Creek Watershed. In this assessment, 16 sites were sampled 12 times and analyzed for *E. coli* and host fecal source identification (cattle versus non-cattle). Flow and nutrient data were also collected 12 times at 6 sites.

In general Stock Creek contained low levels of nutrients in the samples collected at the 6 sites on the main branch. Both total nitrogen and ammonia concentrations were below the detection limit (0.1 mg/L and 0.02 mg/L) in >70% of the samples with the highest reported value for total nitrogen being 0.31 mg/L and the highest value for ammonia being 0.57 mg/L. Nitrite plus nitrate (NO<sub>2</sub> & NO<sub>3</sub>) were detected in all samples with the geometric means across all twelve sample-dates for each site ranging from 0.44 mg/L to 0.61 mg/L. Total phosphorus geometric mean values ranged from 13 ug/L to 23 ug/L, which is below the TDEC limit for total phosphorus of 36.56 ug/L for Ecoregion IX but slightly above the limit for total phosphorus of 10 ug/L for Ecoregion XI. The geometric means for turbidity, which ranged from 5.6 to 9.8 NTU, were below the turbidity limit of 17.5 for Ecoregion X but were above the limits of 5.7 and 2.3 for Ecoregions X and IX, respectively. Geometric means for total organic carbon ranged from 1.7 to 2.6 mg/L and dissolved residues ranged from 166 to 213 mg/L. Dissolved oxygen (9.1 to 10.0 mg/L), pH (7.8 to 8.0), alkalinity (144 to 171 mg/L of CaCO<sub>3</sub>), hardness (164 to 186 mg/L of CaCO<sub>3</sub>) also appeared to be in the normal range.

Across the sampling sites and time, the *E. coli* concentrations ranged from below the recreational water quality limit (126 CFU/100ml) to more than 10-times the recreational water quality (highest value 2400 CFU/100ml). One site (SB-1) completely met the water quality criteria with a geometric mean for *E. coli* of 68 CFU/100ml for all twelve dates and no values for individual samples above 487 CFU/100 ml. Two sites (NM-1 and SC-7) were close to meeting the water quality standards with *E. coli* geometric means of 111 and 129 CFU/100ml, respectively, and only 1 and 2 samples above 487 CFU/100ml, respectively. The other 13 sites had *E. coli* geometric mean values above 200 CFU/100ml and multiple samples above 487 CFU/100ml. Classification of the sampling sites into sub-watersheds indicated that some sub-watersheds had higher amounts of pathogen contamination than other sub-watersheds. Thus identification of the sub-watersheds with the highest pathogen concentrations will aid in the targeting resources for remediation.

*Bacteroides* real-time PCR assays were used to estimate fecal concentrations in all samples and also to discriminate cattle fecal contamination from all other fecal contamination. Discrimination of fecal contamination into cattle versus non-cattle indicated that in the whole watershed 25% of fecal contamination was attributable to cattle. However, there was considerable variability in the amount of the fecal contamination attributable to cattle, with one site having 80% of the fecal contamination



attributable to cattle and four sites having 6% or less of the fecal contamination attributable to cattle. This analysis suggests that BMPs targeting sub-watersheds having high percentages of cattle fecal contamination would reduce pathogen contamination greatly in these areas and also reduce the pathogen load in the total watershed. These results also suggest that implementation of BMPs alone will not reduce pathogen levels to the recreational water quality limit across the whole watershed. At four sites the amount of pathogen contamination attributable to cattle was very low.

In order to better understand the role of water flow on the levels of pathogen and fecal contamination in the watershed, *E. coli* concentrations and fecal concentrations were converted to load data using flow measurements collected at the 6 sites on Stock Creek for all twelve sample dates. The load data was examined with respect to the flow percentile and distance along the main branch. With respect to flow percentile, two types of load duration curves were identified: flow dependent (load increases with increasing flow) and flow independent (load remains constant even with decreasing flow). In general *E. coli* loads attributable to cattle were flow dependent as may be expected from manure runoff. The most notable example for a flow dependent *E. coli* load curve attributable to cattle was the GH-1 site. However, *E. coli* load curves not attributable to cattle (presumptive human fecal contamination) consisted of both dependent and independent types suggesting that *E. coli* contamination from human feces into Stock Creek may occur through multiple routes. An *E. coli* load profile was generated for Stock Creek by plotting the geometric mean of load for each site by distance. In this analysis *E. coli* load increases most across 2 miles between SC-7 and SC-5 and plateaus between SC-3 and SC-1. The relative contributions of *E. coli* load to the main branch by three tributaries were also examined. In this analysis, *E. coli* contributions by the tributaries to the main branch were relatively small ranging from 16% for HB-1 to 3% for GH-1, indicating that although HB-1 and GH-1 had some of the highest *E. coli* concentrations the load contributions are smaller than the main branch because the volume of water is less. The fecal load patterns generated from the *Bacteroides* real-time PCR data were similar to the *E. coli* load patterns with the highest increases in both total fecal loading and cattle-associated fecal loading occurring between SC-7 and SC-5.

A comprehensive source analysis based on *Bacteroides* 16S rRNA gene sequence information was performed to verify cattle sources of contamination and to determine the level of human sources of contamination. Across the watershed, sequences identified with high confidence were predominantly assigned to either humans (63%) or cattle (33%). Another 4% of the sequences were attributed to horses or other animals. When considered on a site-to-site basis, 12 out of 13 sites (92%) had sequences of human fecal origin (14% to 63% of all sequences). In general the sites along the main branch of the creek contained mixtures of human- and cattle- associated *Bacteroides* sequences whereas, the samples from the sites at tributaries contained only human-associated or cattle-associated *Bacteroides* sequences. The GH-1 tributary site was the only site that contained sequences exclusively of cattle origin (GH-1), whereas 4 tributary sites only contained sequences of human origin (NS-1, GV-1, MB-1, HB-1).

## **Overview**

A water quality assessment was performed between April 2003 and February 2004 with the goal of determining the level of pathogen contamination in the Stock Creek Watershed. In this assessment, 16 sites were sampled 12 times and analyzed for *E. coli* and host fecal source identification (cattle versus non-cattle). Flow and nutrient data were also collected 12 times at 6 sites. The research objective of this project was to develop real-time PCR assays for the differentiation and quantification of fecal anaerobic bacteria within the genus *Bacteroides*. Data collected in this research project was expected to provide information regarding the sources of fecal contamination (cattle versus human) necessary for the development of a TMDL for pathogens in the Stock Creek Watershed.

Sixteen sites were selected in the Stock Creek Watershed (Figure 1). These sites were sampled 12 times and were analyzed for bacterial pathogens (Fecal coliform, *E. coli*, and *Enterococcus*) by the Tennessee Department of Health, Knoxville Regional Laboratory (KRL). In addition, nutrients, solids, turbidity, pH, temperature, and flow were determined at six of the sites by KRL. Duplicate samples were taken for pathogen analysis (*E. coli* or *Enterococcus*) and filtered and frozen for real-time PCR analysis and DNA sequence analysis at the Center for Environmental Biotechnology (CEB).

This report is divided into 3 sections as listed below.

### Section I

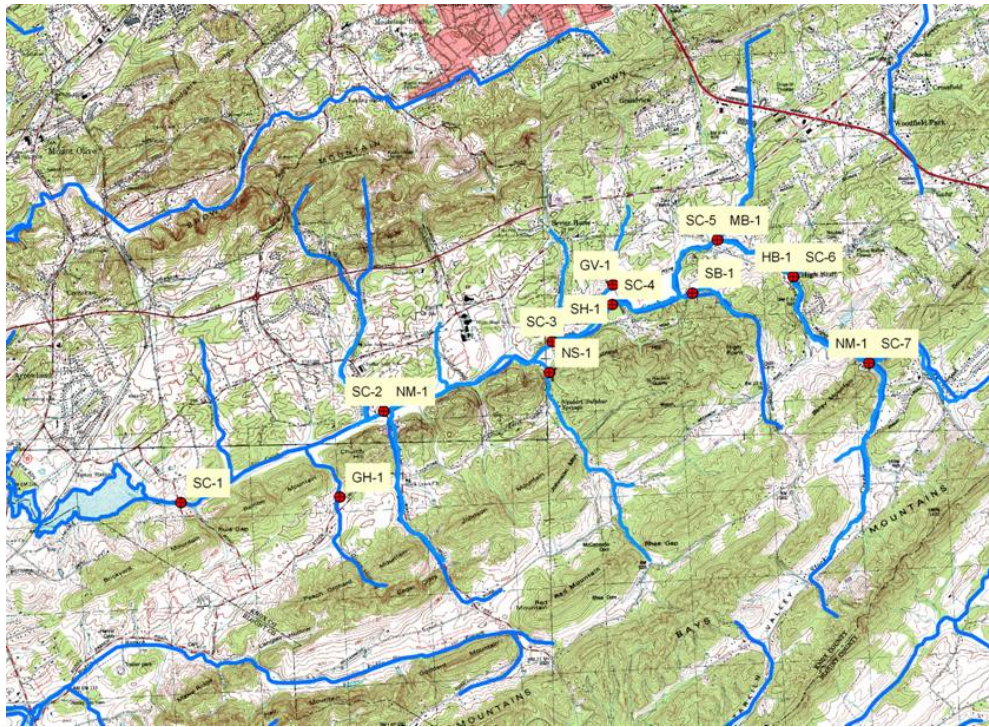
The first section contains summaries of the raw data for bacteriological, ancillary data including nutrients (nitrogen, phosphorus, DO, Turbidity, alkalinity and hardness), and total and bovine fecal concentration as measured by the targeted real-time PCR assay. Data is summarized in tables and figures as concentrations. The bacteriological data is also summarized in tabular form for sub watersheds.

### Section II

The second section contains the relevant data converted into load. This begins with the measured flow data and the steps and assumptions used to extrapolate flow data to tributaries. This section ends with bacteriological data summarized in load duration curves and tables.

### Section III

The third section contains the comprehensive source tracking performed by DNA sequence analysis of the *Bacteroides* 16S rRNA gene. The data is summarized in Tables and also as a pie chart showing the distribution of sequences for the whole watershed identified as Human, Cattle or Other.



**Figure 1.** Stock Creek and associated sample locations.

## **Section I. Summary of Physical, Chemical and Bacteriological Data**

Six sites along the main branch of Stock Creek were analyzed for nutrients (nitrite/nitrate, ammonia, total Nitrogen, phosphorus and total organic carbon), turbidity, pH and DO 12 times during the one-year period. In addition, the 6 sites on the main branch and 10 other sites were analyzed for bacteriological parameters (*E. coli*, *Enterococcus*, *Bacteroides*). Table 1 provides a list of parameters measured between April 2003 and February 2004 and the organization performing the analysis.

Table 1. Descriptions of parameters measured in the Stock Creek Watershed.

Parameter	Description	Units	Assay performed by
Sample	Sample locations using abbreviations listed in Figure 1.	200 to 500 ml volumes collected in appropriate bottles	collected by CEB <sup>a</sup>
Flow	Data collected in Field	CFS	CEB
temp	Data collected in Field	°C	CEB
pH	Data collected in Field	PH units	CEB
conductivity	Data collected in Field	uS	CEB
dissolved O <sub>2</sub>	Data collected in Field	mg/L	CEB
m24- <i>E.coli</i>	Hach assay for <i>E. coli</i> . 1ml, 10 ml, and 100 ml water samples	CFU/100ml (Blue colonies) Average and standard deviation of 3 dilutions	CEB
coliforms	Hach assay for <i>E. coli</i> and coliforms. 1ml, 10 ml, and 100 ml water samples	CFU/100ml (Blue +Red colonies) Average and standard deviation of 3 dilutions	CEB
AllBac Assay	Real-time PCR assay to quantify number <i>Bacteroides</i> 16S operons in samples from all warm blooded animal hosts.	Copies/ml Average and Standard deviation of 3 subsamples from sample	CEB
BoBac Assay	Real-time PCR assay to quantify number of bovine specific <i>Bacteroides</i> 16S operons in samples.	Copies/ml Average and Standard deviation of 3 subsamples from sample	CEB
mf fecal coliforms	Membrane filter for fecal coliforms (44°C) method SM9222D	CFU/100ml Single sample	KRL <sup>b</sup>
colilert	<i>E. coli</i> colilert method SM9223	CFU/100ml Single sample	KRL
enterolert	Enterococcus method SM9223	CFU/100ml Single sample	KRL
alkalinity	Total as CaCO <sub>3</sub> , Method A.1.1	mg/L	KRL
hardness	Total as CaCO <sub>3</sub> , Method A.12	mg/L	KRL
Residue	method A.24.3	mg/L	KRL

dissolved			
Residue suspended	method A.24.2	mg/L	KRL
ammonia	EPA 350.1	mg/L	KRL
NO <sub>3</sub> & NO <sub>2</sub>	EPA 353.2	mg/L	KRL
Total Kjeldahl Nitrogen	EPA 351.2	mg/L	KRL
Total Phosphorus	EPA365.4	mg/L	KRL
Total Organic Carbon (TOC)	EPA 415.1	mg/L	KRL
turbidity	method A.29	NTU	KRL

<sup>a</sup>CEB= Center for Environmental Biotechnology at the University of Tennessee

<sup>b</sup>KRL. Tennessee Department of Health/ Knoxville Regional Laboratory

#### A. Summary of Physical and Chemical Parameter

The geometric means of relevant physical and chemical parameters for twelve sample dates and six Stock Creek sites are provided in Table 2. Temperature is included in a separate table because of the seasonal variability of water temperature (Table 3). The values for ammonia, total nitrogen (Kjeldahl), and suspended residues were not included in the table because the values were either below the detection limit or not reported for a large portion of the data set. For example, ammonia was not detectable in 73% of the samples and only four samples had values above 0.1mg/L. The highest ammonia value was 0.57 mg/L for SC-7 on 1/27/04. For total nitrogen (Kjeldahl), 81% of the samples were below the detection limit and only one sample was above 0.31 mg/L (0.34 at SC-6 on 9/16/03). In this study the NO<sub>2</sub> and NO<sub>3</sub> geometric mean values were higher (0.44 to 0.61 mg/L) than the total nitrogen values.

Total phosphorus geometric mean values ranged from 13 ug/L to 23 ug/L, which is below the TDEC limit for total phosphorus of 36.56 ug/L for Ecoregion IX but slightly above the limit for total phosphorus of 10 ug/L for Ecoregion XI. The geometric means for turbidity, which ranged from 5.6 to 9.8 NTU, were below the turbidity limit of 17.5 for Ecoregion X but were above the limits of 5.7 and 2.3 for Ecoregions X and IX, respectively. Geometric means for total organic carbon ranged from 1.7 to 2.6 mg/L and dissolved residues ranged from 166 to 213 mg/L. Dissolved oxygen (9.1 to 10.0 mg/L), pH (7.8 to 8.0), alkalinity (144 to 171 mg/L of CaCO<sub>3</sub>), hardness (164 to 186 mg/L of CaCO<sub>3</sub>) also appeared to be in the normal range.

Table 2. Geometric means for chemical and physical water quality parameters for 6 sites on Stock Creek.

	pH	Conductivity	DO	Turbidity (NTU)	NO2/NO3 (mg/L)	Total Phosphorus (mg/L) <sup>a</sup>	TOC	Dissolved Residues	Alkalinity	Hardness
SC-2	7.9	213	9.1	9.8	0.44	0.013	2.2	213	171	182
SC-3	8.0	199	9.2	9.7	0.53	0.015	2.6	199	175	186
SC-4	8.0	197	9.3	7.9	0.57	0.015	1.8	197	173	184
SC-5	8.0	181	9.7	6.6	0.60	0.014	2.0	181	165	173
SC-6	7.9	184	9.9	7.0	0.61	0.012	2.1	184	144	167
SC-7	7.8	165	10.0	5.6	0.49	0.023	1.7	165	155	164

Samples with concentrations below the detection limit were assumed to have 0.004 mg/L for purposes of calculating the geometric means.

Table 3. Water temperature at the time of sampling for six Stock Creek sites.

Site	4/30/03	6/4/03	7/9/03	8/13/03	8/26/03	9/16/03	10/09/03	10/30/03	11/20/03	12/11/03	1/27/04	2/19/04
SC-2	17.6	16.7	20.6	19.2	20.2	16.8	15.6	10.5	11.0	8.0	8.5	6.0
SC-3	17.4	16.9	20.9	19.4	20.4	16.6	15.4	10.4	10.8	7.8	8.2	5.8
SC-4	17.5	16.9	21.3	19.6	20.7	16.7	15.4	10.4	10.9	7.7	8.3	6.0
SC-5	17.3	16.9	20.3	18.9	19.9	16.7	15.8	12.3	11.7	8.2	8.7	7.4
SC-6	16.6	15.9	18.8	18.8	18.6	15.8	15.4	NA	12.2	8.8	9.1	9.1
SC-7	17.9	15.6	18.6	18.7	19.0	16.4	15.3	NA	13.4	10.0	10.0	11.1

Table 4. *E. coli* values obtained by Colilert assay. Highlighted values exceed 1000 CFU/100ml.

Date	SC-1	GH-1	SC-2	MM-1	SC-3	SH-1	NS-1	SC-4	GV-1	SB-1	SC-5	MB-1	SC-6	HB-1	SC-7	NM-1
4/30/2003	172	287	388	118	178	328	128	205	76	37	313	147	166	131	91	36
6/4/2003	461	579	488	308	613	770	201	649	921	129	1041	1203	248	1414	44	219
7/9/2003	44	921	365	1120	219	345	219	308	387	96	488	143	770	866	144	79
8/13/2003	517	225	231	250	276	219	79	272	260	131	548	89	129	980	84	83
8/25/2003	105	345	1986	201	649	276	326	184	2419	196	345	115	291	397	326	54
9/16/2003	194	579	173	291	192	517	157	199	228	73	117	199	344	461	91	62
10/9/2003	148	1553	326	153	816	291	158	1733	167	67	185	192		344	137	73
10/30/2003	99	261	206	66	1230	102	1300	2419	308	32	2419	205	211	152	24	86
11/20/2003	1300	613	1120	727	980	411	1046	1986	727	125	1986	649	1553	921	1120	1733
12/20/2003	1414	461	866	488	1300	387	921	1046	1300	85	1414	308	687	291	921	387
1/27/2004	225	173	179	104	185	111	101	197	157	58	435	115	222	326	153	101
2/19/2004	214	579	50	276	59	93	35	135	99	9	184	144	138	194	28	46
GeoMean	245	455	348	251	388	267	224	462	346	68	516	208	313	414	129	111

Table 5. *Enterococcus* concentrations as measured by the enterolert assay.

Date	SC-1	GH-1	SC-2	MM-1	SC-3	SH-1	NS-1	SC-4	GV-1	SB-1	SC-5	MB-1	SC-6	HB-1	SC-7	NM-1
4/30/2003	201	727	285	89	1553	1414	579	613	435	299	613	365	1203	488	219	130
6/4/2003	816	1011	1300	1553	2419	>2419	2419	>2149	>2419	>2419	>2419	>2419	1733	>2419	183	2419
7/9/2003	101	>2419	2419	670	>2419	>2419	>2419	1986	>2419	2419	1300	1300	980	>2419	435	816
8/13/2003	179	1414	1120	436	1553	1046	980	251	1300	251	525	1120	225	2419	197	285
8/25/2003	137	1986	1300	1553	727	2419	1986	1300	2419	1553	1553	1203	488	2419	137	178
9/16/2003	687	2500	770	727	1120	2419	770	479	1986	921	517	921	613	1203	140	104
10/9/2003	249	2419	373	816	921	2500	921	687	1046	921	268	548	111	1300	116	155
10/30/2003	52	548	117	85	214	517	60	80	435	78	51	73	103	231	41	32
11/20/2003	2419	560	2000	2419	2450	1986	2450	2450	1203	921	2450	2419	2450	1553	2450	2450
12/20/2003	2450	1733	2450	2419	2450	2450	2450	2450	980	816	2450	1203	2450	1414	2450	1203
1/27/2004	816	1120	866	727	770	1414	228	1300	1300	1120	488	1120	162	1734	326	548
2/19/2004	24	30	49	280	72	130	60	48	37	5	47	35	69	120	91	13
GeoMean	294	872	676	628	899	1259	655	604	767	423	512	584	466	928	249	269

B. Bacteriological Monitoring (*E. coli* and *Enterococcus*).

*E. coli* was measured by the Colilert method at KRL for all samples and at CEB by the Hach Filter Membrane assay method for 144 samples. The *E. coli* values obtained by the Colilert and Hach Filter Membrane assays were well correlated ( $r=0.81$ ). However, since the Colilert values represented a complete data set they are the only values reported in this report (Table 3). One site (SB-1) completely met the water quality criteria with a geometric mean for *E. coli* of 68 CFU/100ml for all twelve dates and no values for individual samples above 487 CFU/100 ml. Two sites (NM-1 and SC-7) were close to meeting the water quality standards with *E. coli* geometric means of 111 and 129 CFU/100ml, respectively, and only 1 and 2 samples above 487 CFU/100ml, respectively. The other 13 sites had *E. coli* geometric mean values above 200 CFU/100ml and multiple samples above 487 CFU/100ml.

*Enterococcus* values were also determined at KRL using the Enterolert assay as shown in Table 4. The correlation between *E. coli* and *Enterococcus* concentrations for all samples was  $r = 0.44$ .

C. Real-Time PCR assays: Targeted evaluation of total *Bacteroides* and Bovine-Specific *Bacteroides*

Real-time PCR assays for all *Bacteroides* (AllBac) and bovine-specific *Bacteroides* (BoBac) were applied to samples collected at 16 Stock Creek sites on 12 different sampling dates. In addition, an *E. coli* real-time PCR assay was performed for each sample so that the concentrations of *E. coli* and *Bacteroides* could be compared using the same methodology. Data was initially calculated as gene copies per 100 mls. *Bacteroides* rRNA genes were found in all samples and may have resulted from several different sources including human, cattle, wildlife and other domesticated animals such as horses, dogs or cats. In all sites *Bacteroides* rRNA concentrations were higher than the *E. coli* rRNA concentrations by at least 100 fold (Figure 4). This is not surprising because *Bacteroides* rRNA genes are considerably higher in fecal samples than *E. coli* rRNA genes. Cattle associated-*Bacteroides* were generally lower and more variable than all *Bacteroides* with some samples at or below the detection limit especially in samples from the tributaries. However, one particular site (GH-1) had very high levels (1000 fold above the detection limit) of cattle-specific *Bacteroides* between 4/30/03 and 11/20/03 suggesting that the dominant source of fecal contamination at this site was of bovine origin. The high cattle-specific *Bacteroides* at the GH-1 site is also reflected in the Geometric means as shown in Figure 4. Sites with very low cattle-specific *Bacteroides* included HB-1, GV-1, SB-1 and MB-1.

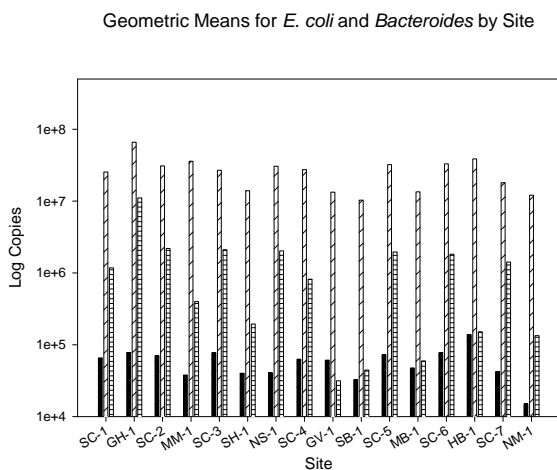


Figure 4. Geometric means (copies/100ml) for each real-time PCR assay for 12 sampling periods for each stock creek site. Solid bars represent *E. coli* concentrations, diagonal striped bars represent total *Bacteroides* concentrations (AllBac assay) and horizontal striped bars represent bovine associated *Bacteroides* concentrations (BoBac assay).



Temporal Variability

The geometric means for the *E. coli* and real-time PCR data were calculated across all sites for each sample data to determine the extent of variability in the data set with respect to time (Figure 5). In this analysis the geometric means of both the AllBac assay and *E. coli* CFU/100ml exhibited a ten-fold range with the highest values occurring in fall 2003 and the lowest values occurring in winter 2004. In addition, the AllBac copies/100ml and *E. coli* CFU/100ml showed the same trends and were correlated ( $r=0.72$ ) suggesting these differences were not simply due to measurement error. However, at this time it is not clear as to whether these difference reflect changes in seasons, water flow or land-use conditions.

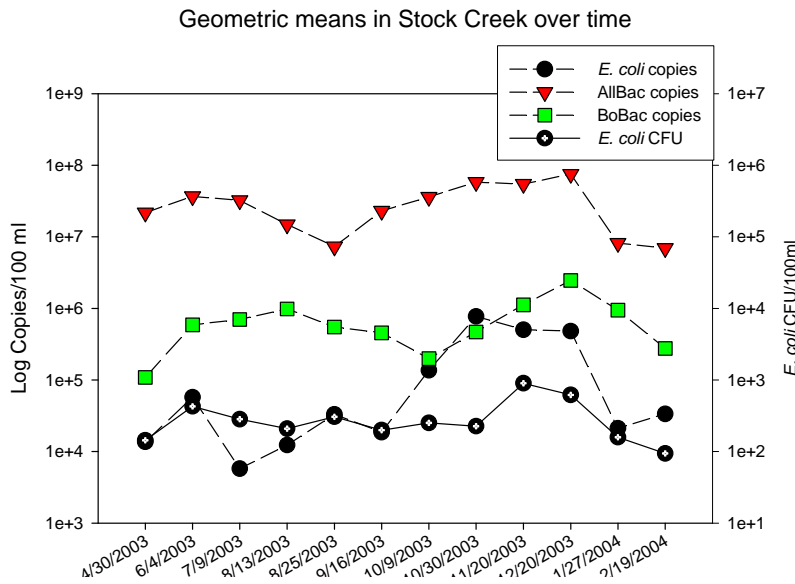


Figure 5. Geometric means of real-time PCR data (*E. coli* copies, AllBac copies, BoBac copies) and *E. coli* concentrations (CFU/100 ml) in Stock Creek for all samples on a given sampling date.

Conversion of Real-time PCR data from Copies/100mls to mg/L feces

In this study, the copies of *Bacteroides* 16S rRNA genes/100 mls were converted to mg/L feces assuming that feces contain approximately  $2 \times 10^{10}$  copies/gram of any feces using the AllBac assay and  $5 \times 10^9$  copies/gram of bovine feces using the BoBac assay (Layton et al., 2006. Manuscript in review). This conversion factor has been revised from earlier reports, which used the values  $2 \times 10^{11}$  and  $5 \times 10^{10}$  copies for all feces and bovine feces, respectively based on more sample data. The mg/L values for all feces and bovine feces and the geometric means and averages are presented in Tables 6 and 7. The percentage of feces in each sample attributable to cattle was calculated for each sample by dividing the bovine specific feces (mg/L) by the total feces (mg/L) and multiplying by 100 (Table 8). The percentage of feces attributable to cattle for each site was summarized by calculating the geometric mean and average across all the twelve sample dates. The average percentage of feces attributable to cattle for all samples (dates and sites) was 25%.

Table 6. Total fecal concentrations (mg/L) in Stock Creek as determined by the AllBac real-time PCR assay.

	SC-1	GH-1	SC-2	MM-1	SC-3	SH-1	NS-1	SC-4	GV-1	SB-1	SC-5	MB-1	SC-6	HB-1	SC-7	NM-1
4/30/2003	17.97	197.95	14.14	13.92	15.22	8.48	12.01	9.20	5.17	4.91	15.51	4.12	12.72	11.99	4.95	2.91
6/4/2003	24.94	95.78	21.39	32.07	21.98	15.18	13.58	22.33	13.99	13.33	20.45	17.21	17.46	27.69	6.64	4.62
7/9/2003	1.15	235.30	15.59	32.20	13.57	13.64	37.00	22.99	12.37	22.88	22.24	3.78	23.00	21.78	12.69	6.32
8/13/2003	9.40	17.29	10.18	27.63	9.22	6.87	5.39	10.66	2.85	3.19	10.44	6.88	5.57	14.23	4.42	2.25
8/25/2003	6.83	42.25	25.12	34.08	27.57	10.33	34.81	23.44	14.64	9.10	41.96	13.86	45.87	23.37	17.31	6.73
9/16/2003	18.34	31.36	16.60	18.62	9.85	7.19	21.78	12.12	6.25	6.67	17.27	7.22	26.18	10.87	4.89	3.92
10/9/2003	29.20	47.10	28.48	45.29	24.53	8.11	33.23	13.11	10.66	6.93	21.70	6.45	38.77	16.24	12.63	9.11
10/30/2003	41.09	42.14	34.82	66.15	23.23	22.29	28.92	27.79	15.39	16.69	34.52	18.04	47.29	26.31	19.38	38.53
11/20/2003	39.40	35.05	38.91	24.89	35.46	14.53	53.32	28.23	14.85	8.51	30.96	23.02	32.51	35.47	17.19	48.09
12/20/2003	74.48	30.32	75.90	41.34	61.49	17.57	107.74	44.34	14.16	6.95	59.50	24.68	45.40	62.80	32.15	32.15
1/27/2004	4.99	6.00	4.37	5.29	3.23	2.67	4.55	4.14	1.91	0.57	5.09	1.75	3.36	19.32	9.63	2.03
2/19/2004	5.14	5.97	3.10	4.22	4.88	1.61	3.85	3.63	2.56	0.91	5.66	1.84	3.89	18.09	2.69	2.14
GeoMean	13.96	37.49	17.45	22.56	15.69	8.66	19.12	14.65	7.64	5.64	18.93	7.64	18.07	21.29	9.51	6.91

Table 7. Bovine-specific fecal concentrations (mg/L) in Stock Creek as determined by the BoBac real-time PCR assay.

	SC-1	GH-1	SC-2	MM-1	SC-3	SH-1	NS-1	SC-4	GV-1	SB-1	SC-5	MB-1	SC-6	HB-1	SC-7	NM-1
4/30/2003	2.34	67.68	1.92	0.01	2.16	0.27	1.13	0.58	0.01	0.01	1.09	0.01	1.31	0.03	0.36	0.00
6/4/2003	6.75	37.78	3.56	0.29	3.92	2.45	1.64	1.12	0.04	0.04	10.52	0.23	2.42	0.52	1.97	0.30
7/9/2003	0.01	357.40	8.57	0.45	6.53	4.51	11.89	6.24	0.51	0.10	8.18	0.11	7.72	0.10	5.92	0.04
8/13/2003	5.84	16.78	3.66	39.81	9.60	0.73	4.14	4.60	0.04	0.03	3.78	0.71	3.77	1.52	4.29	0.39
8/25/2003	6.27	44.57	4.15	1.19	0.64	0.40	16.03	1.66	0.05	0.10	7.79	0.01	9.73	0.38	8.25	0.08
9/16/2003	2.25	23.03	2.30	1.47	1.46	1.44	5.60	0.94	0.07	0.17	1.98	0.10	2.71	0.11	1.19	0.13
10/9/2003	2.65	25.11	6.71	0.07	9.10	0.10	1.17	0.09	0.08	0.03	1.40	0.05	1.34	0.02	1.20	0.03
10/30/2003	2.18	41.70	8.00	3.68	9.74	1.78	0.25	0.45	0.34	0.02	2.57	0.07	2.08	0.05	2.28	0.28
11/20/2003	7.64	1.58	10.90	6.33	7.88	0.01	18.12	6.44	0.18	0.32	7.31	0.61	9.53	0.66	3.99	13.88
12/20/2003	7.50	5.56	9.56	5.26	8.75	1.05	20.70	11.44	0.25	1.38	12.04	1.55	8.63	1.84	20.70	11.64
1/27/2004	4.13	10.58	2.38	0.78	2.46	0.59	7.40	2.44	0.01	0.04	2.74	0.71	3.89	4.46	3.59	1.33
2/19/2004	1.97	9.18	1.85	0.09	4.15	0.01	2.88	1.70	0.03	0.01	2.66	0.04	2.45	1.61	1.89	0.44
GeoMean	2.35	22.17	4.38	0.80	4.21	0.39	4.04	1.63	0.06	0.06	3.90	0.12	3.64	0.30	2.84	0.27

Table 8. Percentage of feces attributable to cattle for each Stock Creek site and sample date.

	SC-1	GH-1	SC-2	MM-1	SC-3	SH-1	NS-1	SC-4	GV-1	SB-1	SC-5	MB-1	SC-6	HB-1	SC-7	NM-1
4/30/2003	13.0	34.2	13.6	0.1	14.2	3.1	9.4	6.3	0.1	0.1	7.0	0.2	10.3	0.2	7.3	0.1
6/4/2003	27.1	39.4	16.6	0.9	17.9	16.1	12.1	5.0	0.3	0.3	51.4	1.3	13.9	1.9	29.7	6.5
7/9/2003	0.7	151.9	55.0	1.4	48.1	33.1	32.1	27.2	4.1	0.4	36.8	28	33.6	0.5	46.6	0.7
8/13/2003	62.1	97.1	36.0	144.1	104.1	10.6	76.8	43.1	1.2	0.9	36.2	10.3	67.6	10.7	97.2	17.4
8/25/2003	91.8	105.5	16.5	3.5	2.3	3.8	46.1	7.1	0.3	1.1	18.6	0.0	21.2	1.6	47.7	1.2
9/16/2003	12.3	73.4	13.8	7.9	14.9	20.0	25.7	7.8	1.1	2.6	11.4	1.4	10.4	1.0	24.4	3.2
10/9/2003	9.1	53.3	23.6	0.2	37.1	1.3	3.5	0.7	0.7	0.5	6.5	0.7	3.5	0.1	9.5	0.3
10/30/2003	5.3	99.0	23.0	5.6	41.9	8.0	0.8	1.6	2.2	0.1	7.4	0.4	4.4	0.2	11.8	0.7
11/20/2003	19.4	4.5	28.0	25.4	22.2	0.0	34.0	22.8	1.2	3.7	23.6	2.7	29.3	1.9	23.2	28.9
12/20/2003	10.1	18.3	12.6	12.7	14.2	6.0	19.2	25.8	1.8	19.9	20.2	6.3	19.0	2.9	64.4	36.2
1/27/2004	82.7	176.3	54.4	14.7	76.2	22.0	162.7	59.0	0.4	6.3	53.8	40.3	115.7	23.1	37.2	65.3
2/19/2004	38.4	153.9	59.5	2.1	85.1	0.9	75.0	46.7	1.3	0.9	46.9	2.0	63.1	8.9	70.3	20.8
<u>GeoMean</u>	17	59	25	4	27	4	21	11	1	1	21	2	20	1	30	4
average	31	84	29	18	40	10	41	21	1	3	27	6	33	4	39	15

D. Spatial distribution of *E. coli* and Percentage of feces attributable to bovine

Analysis of the bacteriological data indicated that although 25% of the fecal contamination in the whole watershed was attributable to cattle, there was spatial variability in *E. coli* concentration, total fecal concentration and the presence of cattle fecal contamination. In order to identify the areas of the watershed with the highest pathogen contamination the watershed was divided into sub-watersheds as shown in Table 9. Several of the sub-watersheds contained a tributary sampling site along with a sampling site along the main creek (SC samples). Three of the sub-watersheds did not have any sample sites. In Table 9, the percentage of samples with values greater than or less than 126 CFU/100 was first determined from Table 4 for each site in the sub-watershed. In addition the percentage of feces attributable to cattle was derived from the average percentage of bovine feces for each site from Table 8.

These results indicate that three sub-watersheds (01, PG, and SB-1) have low levels of *E. coli* contamination. However, most of the sub-watersheds have moderate amounts of *E. coli* contamination with geometric means in the 200 to 550 CFU/100ml range. The sub-watersheds with the highest impact from cattle appeared to be 07, NS-1 and 01.

Table 9. Pathogen Assessment of Sub-Watersheds in the Stock Creek Watershed

Sub-Watershed	Sampling Site (s)	Pathogen Assesment (The <i>E. coli</i> recreational limit is 126 CFU/100ml)
01	SC-7	66% of samples <b>below</b> recreational limit (Geomean=129), 40% attributable to cattle
PG	NM-1	75% of samples <b>below</b> recreational limit (Geomean=111), 15% attributable to cattle
02	SC-6, HB-1	83% of samples <b>above</b> recreational limit (Geomean= 313), 33% attributable to cattle 100% of samples <b>above</b> recreational limit (Geomean= 414), 4% attributable cattle
ML	SC-5 MB-1	83% of samples <b>above</b> recreational limit (Geomean=516), 27% attributable to cattle 63% of samples <b>above</b> recreational limit (Geomean=208), 6% attributable cattle
03	SB-1	75% of samples <b>below</b> recreational limit (Geomean= 68) , 3% attributable cattle
04	SC-4 GV-1	92% of samples <b>above</b> recreational limit (Geomean=462), 21% attributable to cattle 83% of samples <b>above</b> recreational limit (Geomean=346), 1% attributable to cattle
NS	NS-1	75% of samples <b>above</b> recreational limit (Geomean=224), 41% attributable to cattle
05	No sites	Not tested

CH	No sites	Not tested
06	SC-2	92% of samples <b>above</b> recreational limit (Geomean=348), 29% attributable to cattle
	MM-1	83% of samples <b>above</b> recreational limit (Geomean=251), 18% attributable to cattle
TW	No sites	Not tested
07	GH-1	100% of samples <b>above</b> recreational limit (Geomean= 455), 84% attributable to cattle
CB	No Sites	Not tested
08	SC-1	75% of samples <b>above</b> recreational limit (Geomean 245), 31% attributable to cattle

## **Section II. Calculation of Stock Creek *E. coli* loads and partitioning of *E. coli* loads in to that attributable to bovine using Bruce Cleland's Flow duration Curve Models**

In addition to the chemical, physical and bacteriological measurements, flow (cfs) was measured at six sites on 12 sample dates. In this section biological and chemical parameters were converted to load based on the concentration data for each parameter provided in Section I and flow data (either measured or extrapolated). The methods used for extrapolating flow are described first in this section.

### ***Calculation of Flow Duration Curves***

Flows (cfs) and percentile values for flow duration curves were calculated using the "Flow Duration Tool (Template)" Excel spreadsheet provided by Bruce Cleland (America's Clean Water Foundation). Flow duration curves were presented in Power Point files also provided by Bruce Cleland.

The flow duration curve analysis as presented by Bruce Cleland was originally designed for gauged streams with data available from USGS (<http://waterdata.usgs.gov/nwis/>). However, there is a lack of gauged streams in east Tennessee. The Stock Creek Watershed has one gauge (Pickens Gap), but this gauge only measures stream height and data is not available to calculate flow.

Several people have speculated that general flow duration curves can be created from known data that will be applicable to other streams in a geographic region. This hypothesis was tested by creating flow duration curves for 13 gauged data sets from the Lower Clinch Watershed (USGS 06010207). This watershed was chosen because it is geographically close and geographically similar to the Stock Creek Watershed and because data was available for a number of streams with very small drainage areas. The gauged data sets used are summarized in Table 1. In this watershed stream gauge data were excluded based on the following criteria: 1) very large drainage areas (>100 sq. miles), 2) gauges near dammed areas, 3) gauges with very high flow for the drainage area (EF popular creek). Flow (cfs) was graphed versus Flow Duration Interval (%) in Power Point figures (electronic version available). In addition, a regression analysis was performed across the 13 data sets in Excel comparing the log of the drainage and the log of each Flow Duration Intervals (1-100%) (Table 2). The drainage area and flow was highly correlated ( $r^2 > 0.9$ ) at the high flow to mid flow ranges (1% to 50%). The correlations between flow and drainage area decreased with increasing percentile to  $r^2 = 0.60$  at dry conditions (100%). These results suggest that Linear Regression formulas may be used to predict flows in un-gauged watersheds in geologically and geographically similar areas based on the drainage area of stream. It is expected that these curves will be reliable in the regions of the graph representing moderate to high flows. However, the ability to reliably predict the flow in small streams under very low flow conditions is questionable.

Table 10. Data sets from stream gauges used in this study from the Lower Clinch River Watershed.

Stream	Drainage area (sq.miles)	Gauge Number	Dates of Operation	Number of Sample Points
BULLRUN CREEK NEAR HALLS CROSSROADS, TN	68.5	03535000	1957-2003	11414
POPLAR CREEK NEAR OAK RIDGE, TN	82.5	03538225	1960-1989	10622
BEAR C AT ST HWY 95 NR OAK RIDGE, TN	4.34	03538270	1985-2000	5745
BEAR CREEK NEAR WHEAT, TN	3.2	035382673	1986-1991	1826
BEAR CREEK AT PINE RIDGE, NEAR WHEAT, TN	5.0	03538273	1986-1991	1832
WHITEOAK CREEK NEAR WHEAT, TN	2.10	03536380	1986-1995	3226
NORTHWEST TRIBUTARY NEAR OAK RIDGE, TN	0.67	03536440	1987-1995	3093
FIRST CREEK NEAR OAK RIDGE, TN	0.33	03536450	1987-1996	3530
WHITEOAK CREEK AT O R N L, NEAR OAK RIDGE, TENN	2.08	03536500	1950-1955	1870
WHITEOAK CR BL MELTON VALLEY DR NR OAK RIDGE	3.28	03536550	1985-2001	5698
WHITEOAK CR BL OAK RIDGE NATL LAB NR OAK RIDGE, TN	3.62	0353700	1950-1964	4383
MELTON BRANCH NEAR OAK RIDGE, TN	1.48	03537500	1955-1964 start at 1956	3226
MELTON BRANCH NR MELTON HILL NR OAK RIDGE, TN	0.52	03537100	1985-1995	3844

Table 11. Summary of linear regression for the log flow duration intervals versus log drainage areas in the Lower Clinch Watershed for each Percentile

	1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	99	100
r <sup>2</sup>	1.00	0.99	0.99	0.98	0.98	0.97	0.96	0.96	0.95	0.93	0.91	0.89	0.87	0.85	0.81	0.81	0.79	0.76	0.73	0.71	0.66	0.60
y(0)	1.25	0.78	0.57	0.44	0.34	0.24	0.16	0.08	0.01	-0.07	-0.16	-0.23	-0.29	-0.36	-0.47	-0.52	-0.39	-0.44	-0.53	-0.68	-0.88	-0.79
m	1.02	1.01	1.01	1.01	1.02	1.02	1.03	1.04	1.05	1.05	1.06	1.05	1.05	1.05	1.09	1.06	0.86	0.83	0.82	0.85	0.85	0.68

Table 12. Summary of Percentile flow (cfs) for 9 Stock Creek sites calculated using the linear regression values obtained for each Percentile (Table 2) and the drainage area.

Site	area	1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	99	100
SC-2	14.15	262	88.3	53.6	40	32.2	26	22	19	16.3	13.7	11.6	9.66	8.24	7.1	6.1	5.08	3.92	3.27	2.64	1.97	1.23	0.98
SC-3	8.68	159	53.9	32.8	25	19.6	16	13	11	9.76	8.22	6.91	5.77	4.93	4.25	3.58	3.03	2.58	2.18	1.76	1.3	0.82	0.70
SC-4	7.4	135	45.9	27.9	21	16.7	14	11	9.6	8.26	6.95	5.84	4.88	4.17	3.59	3.01	2.55	2.25	1.91	1.55	1.13	0.71	0.63
SC-5	4.58	83	28.3	17.2	13	10.2	8.3	6.9	5.8	5	4.19	3.51	2.94	2.52	2.17	1.79	1.53	1.49	1.28	1.04	0.76	0.48	0.45
SC-6	4.13	75	25.5	15.5	12	9.22	7.5	6.2	5.2	4.48	3.76	3.15	2.64	2.26	1.95	1.6	1.38	1.36	1.18	0.96	0.69	0.44	0.42
SC-7	1.62	29	9.89	6.05	4.5	3.56	2.9	2.4	2	1.68	1.41	1.17	0.98	0.85	0.73	0.58	0.51	0.61	0.54	0.44	0.31	0.2	0.22
GH-1	0.44	7.7	2.65	1.63	1.2	0.95	0.8	0.6	0.5	0.43	0.36	0.29	0.25	0.22	0.18	0.14	0.13	0.2	0.18	0.15	0.1	0.07	0.09
HB-1	0.47	8.2	2.83	1.74	1.3	1.01	0.8	0.7	0.5	0.46	0.38	0.31	0.27	0.23	0.2	0.15	0.14	0.21	0.19	0.16	0.11	0.07	0.10
NS-1	2.42	44	14.8	9.06	6.8	5.35	4.3	3.6	3	2.56	2.14	1.78	1.5	1.29	1.11	0.89	0.78	0.86	0.76	0.62	0.44	0.28	0.29



The linear regression values presented in Table 2 were used to create presumptive percentile flows for 7 sites on the main creek in the Stock Creek Watershed (Table 3, PowerPoint File). Flow duration curves for each Stock Creek site were generated from the percentile ranks shown in Table 12. Two flow duration curves are shown in Figure 6. Because the values used to generate the flows at each percentile rank are dependent on drainage size, the flow duration curves for other sites, including tributaries, are similar to the two shown differing only in absolute values.

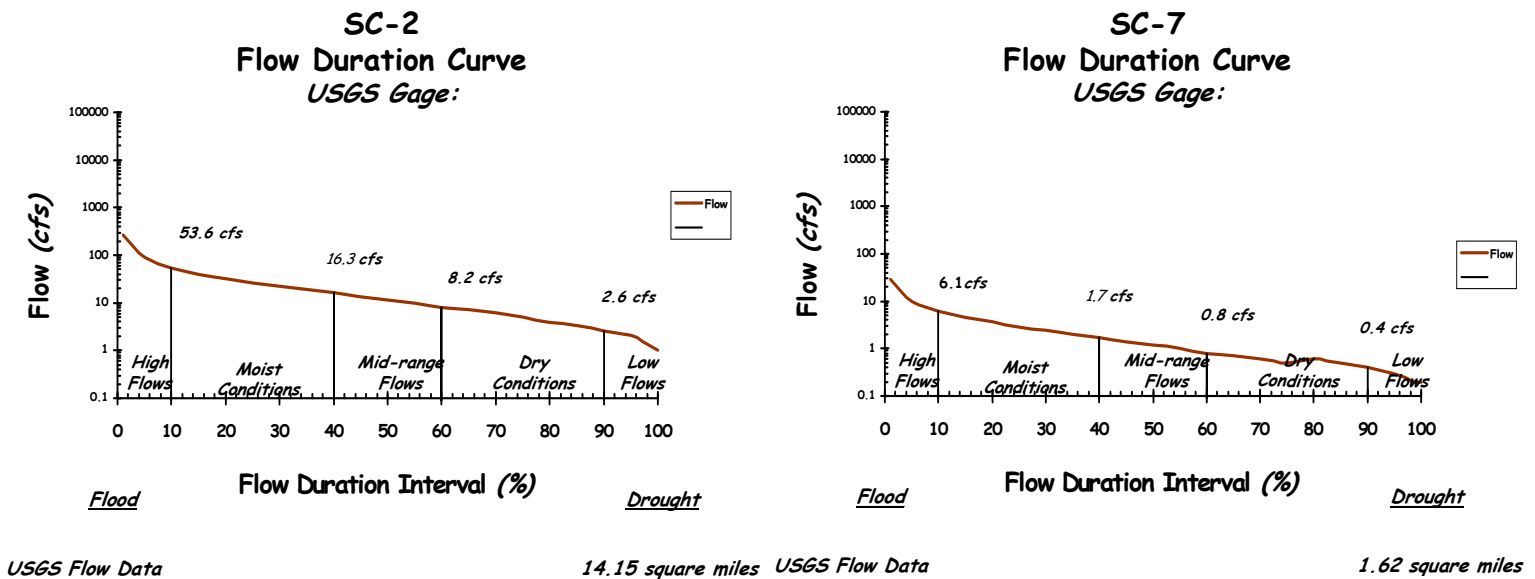


Figure 6. Flow duration curves for SC-2 and SC-7.

The percentile rank for flow on any sampling date can be estimated by comparing the measured flow (CFS) with the percentile rank generated from the flow duration curve. During the 1 year sampling period flows were measured and calculated for 6 sites 12 times (Table 4). The percentile rank for each flow measurement was estimated to the nearest 5% (Table 5) based on the percentile calculations shown in Table 3. Assuming that the relative percentile rank at each site should be similar on any sample date a mean percentile rank was calculated for each date and the flow for the whole watershed was classified as High, Moist, Mid-Range, Dry or Drought. Based on these analyses, the flows were classified as Moist for 7 sample dates, 3 were classified as Mid-Range and 2 were classified as Dry. Samples were taken during very high flows or very low flows (drought).

Table 13. Flow measurements (cfs) by date at 6 sites in the Stock Creek Watershed

Site	4/30/2004	6/4/2003	7/9/2003	8/13/2003	8/26/2003	9/16/2003	10/9/2003	10/30/2003	11/20/2003	12/11/2003	1/27/2004	2/19/2004
SC-2	24.4	15.02	36.23	43.79	8.24	16.37	6.26	5.23	28.25	8.24	23.17	18.4
SC-3	13.05	11.6	23	34.11	5.67	11.68	3.93	3.48	19.21	5.67	11.25	10.89
SC-4	14.32	7.1	27.4	8.64	4.24	9.75	2.99	2.175	13.42	4.24	11.16	8.83
SC-5	12.29	5.57	32.9	5.71	3.06	6.9	2.17	1.59	8.79	3.06	7.55	10.18
SC-6	6.29	3.8	9.8	5.37	2.56	3.92	1.93	1.59	6.5	2.56	5.93	4.83
SC-7	4.16	2.1	11.23	2.08	0.86	2.35	0.754	0.727	2.79	0.87	3.25	2.59

Table 14. Estimation of flow percentile based on presumptive flow duration curves calculated for each site.

Sample Date	Site						Mean	Range	
	SC-2	SC-3	SC-4	SC-5	SC-6	SC-7			
4/30/2003		25	30	25	15	30	15	23	Moist
6/4/2003		40	50	50	59	45	35	47	Mid Range
7/9/2004		25	15	10	5	20	5	13	Moist
8/13/2003		15	10	40	35	35	35	28	Moist
8/26/2004		60	55	60	55	55	60	58	Mid Range
9/16/2004		25	35	35	30	45	30	33	Moist
10/9/2003		70	70	70	65	65	65	68	Dry
10/30/2003		75	70	80	75	70	65	73	Dry
11/20/2003		25	20	30	25	30	25	26	Moist
12/11/2003		60	55	60	55	55	60	58	Mid Range
1/27/2004		30	35	30	30	30	20	29	Moist
2/19/2004		35	35	20	20	40	30	30	Moist

## Calculation of Load Duration Curves for *E. coli*

At any site in the watershed, the load for any parameter can be calculated if both the concentration of the parameter and the flow are known. In the Stock Creek study, *E. coli* concentrations were determined for each site for twelve sample dates (Table 4). Therefore, load can be calculated for each of these data points using measured flow for SC-2 through SC-7 and extrapolated flow for other sites. *E. coli* loads on the main branch of Stock Creek were calculated using the following formula present in the WQ Duration Tool (Template) spreadsheet: Load (CFU/day) = CFU/100ml \* Flow (CFS)\* (28317/100)\*60\*60 \*24.

*E. coli* load duration curves for SC-2 through SC-7 on the main creek and 3 sites on tributaries were also calculated using the flow data for each percentile ranks as shown in Table 12. An *E. coli* load duration curve was generated in the WQ Duration Tool (Template) Excel Spreadsheet assuming acceptable water quality value of 126 *E. coli* CFU/100 ml. These curves were graphically presented in Power Point and *E. coli* load duration curves for SC-2 is shown in Figure 7. The *E. coli* load duration curves for the other sites are similar but differ in absolute values because the *E. coli* value is constant, but the flow at each percentile is based on the drainage area. These types of graphs also indicate that higher amounts of *E. coli* can be carried in water at high flows than at low flows.

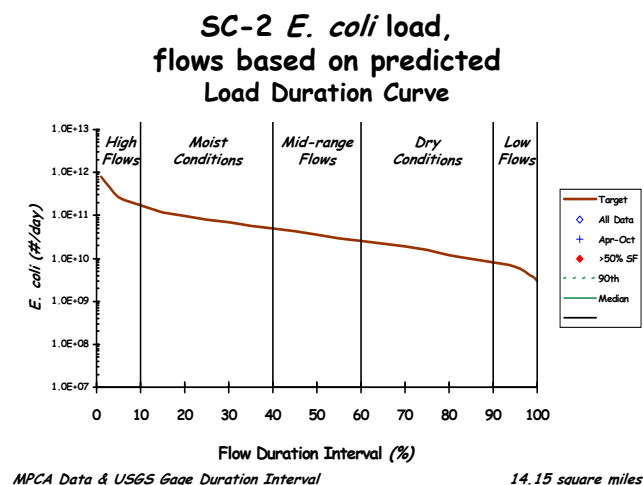


Figure 7. *E. coli* load duration curve calculated using *E. coli* concentrations of 126 CFU/100ml and flow for each percentile as shown in Table 12 and displayed in Figure 6.

When the following three variables: flow (CFS), flow percentile (Flow duration Interval%) and *E. coli* concentration (CFU/100m) for any sample date and site are known, then the *E. coli* load for that sample can be added to the Load Duration curve to determine whether it is over or under the *E. coli* load limit for that flow duration interval and look for seasonal trends. The *E. coli* load for each sample data at each site was calculated using WQ Duration Tool (Template) spreadsheet, using the measured flow and the measured *E. coli* CFU/100 ml. These load values were plotted against Percentile rank on the load duration curves in Power Point. Filled diamonds represent warm weather sample dates (April- October) and unfilled diamond represent cool weather sample dates (November-March). Diamonds above the load duration line represent samples above the equivalent 126 CFU/100ml threshold. In this analysis, sites were identified with respect to the number of data points above the *E. coli* load threshold and also based on the *E. coli* load pattern with respect to the flow (Table 15). Two types of *E. coli* load patterns were identified: flow dependent and flow independent, as shown in Figure 8. Flow independent *E. coli* load patterns would suggest constant *E. coli* source independent of

rainfall or flow conditions. Failing septic tanks, straight pipes or leaking sewer systems are *E. coli* sources that may fit this pattern. In contrast flow dependent *E. coli* load patterns would be more characteristic of *E. coli* entering the stream through runoff. There also was no apparent seasonal trend for the *E. coli* load at any sites.

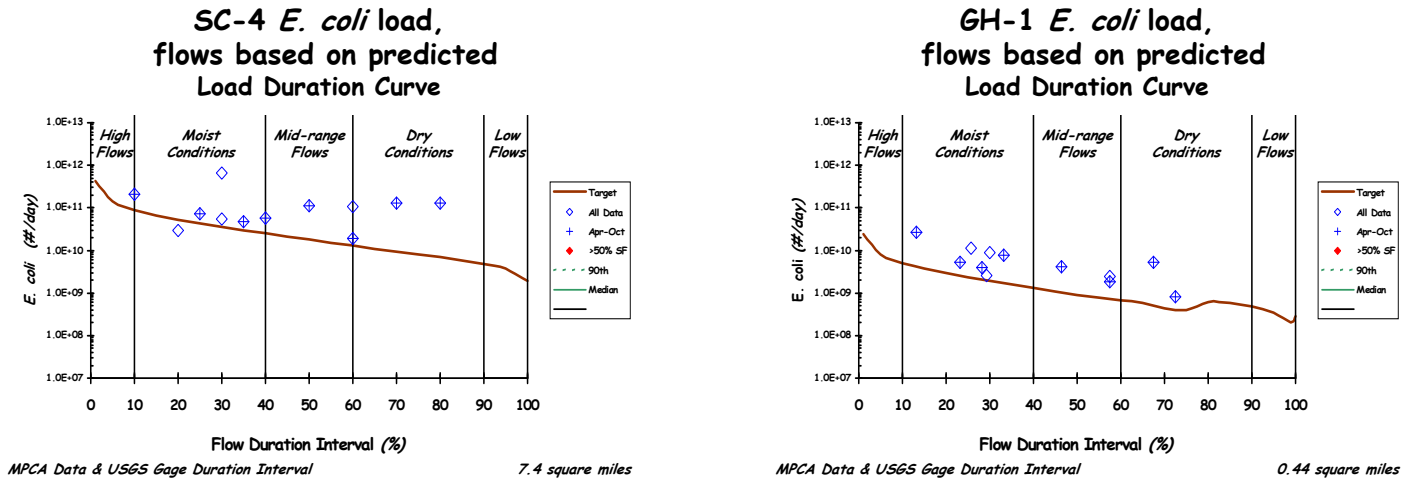


Figure 8. An example of a flow independent *E. coli* Load Pattern (SC-4) and a flow dependent *E. coli* load pattern (GH-1).

Table 15. Characterization of *E. coli* load in Stock Creek based on the Percentage of the samples above the *E. coli* Load Duration Curve and the type of load pattern.

Site	Percent of Samples Above <i>E. coli</i> Load Duration Curve	Sample Load Pattern (Flow dependent, Flow independent)
SC-2	83%	Dependent
SC-3	92%	Independent
SC-4	92%	Independent
SC-5	92%	Independent
SC-6	100%	Dependent
SC-7	25%	Dependent
GH-1	100%	Dependent
NS-1	75%	Independent
HB-1	100%	Dependent

Because the Flow Dependent *E. coli* Load pattern may be due to manure runoff, an attempt was made to use the percentage of feces attributable to bovine sources (Table 8) as a way to separate the *E. coli* Load for each samples into bovine *E. coli* load and non-bovine *E. coli* load. In this analysis, the percentage of feces attributable to bovine was multiplied by the *E. coli* load to determine the *E. coli* load attributable to cattle (percentage values >100% were assumed to be 100). The bovine *E. coli* load was subtracted from the total *E. coli* load to determine the non-bovine *E. coli* load (presumably mostly human). The implicit assumptions in this analysis are that all animal fecal sources have equivalent concentrations of *E. coli* and that *E. coli*

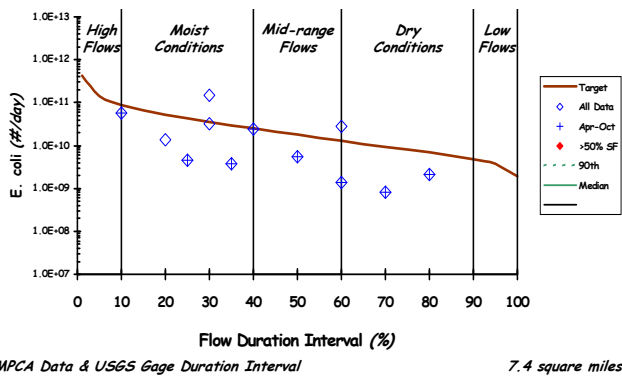
concentration is proportional to *Bacteroides*. In this study, the correlation of all of the AllBac assay values (mg/L) with all *E. coli* concentrations was 0.31 suggesting that *E. coli* concentrations and *Bacteroides* concentrations are loosely correlated. *E. coli* loads attributable to cattle and not attributable to cattle were displayed using Power Point graphics. The sites were then classified with respect to the percentage of samples above the *E. coli* load threshold and the *E. coli* load pattern (Flow dependent or Flow independent) (Table 16). Examples of changes seen in *E. coli* load and *E. coli* load patterns after the *E. coli* load was separated into bovine and non-bovine load are shown in Figure 9. The *E. coli* load attributable to cattle made a large contribution to the total *E. coli* load except at the HB-1 site (Figure1). At two sites SC-5 and GH-1, 50% and 75% of the *E. coli* attributable to cattle loads alone were above the 126 CFU/100 ml threshold, suggesting that removal of the *E. coli* attributable to cattle at these sites would reduce the total *E. coli* load to acceptable limits. In contrast, at the HB-1 site none of the sample dates had *E. coli* loads attributable to cattle above the threshold and 3 of the *E. coli* loads were below the  $1 \times 10^7$  graphing limit. This suggests that at this site removal of *E. coli* attributable to cattle would have little impact on the total *E. coli* loads. Therefore, the *E. coli* loads at this site must be due to another source such as human.

Table 16. Characterization of bovine associated *E. coli* load and non-bovine associated load in Stock Creek based on the Percentage of the samples above the *E. coli* Load Duration Curve and the type of load pattern.

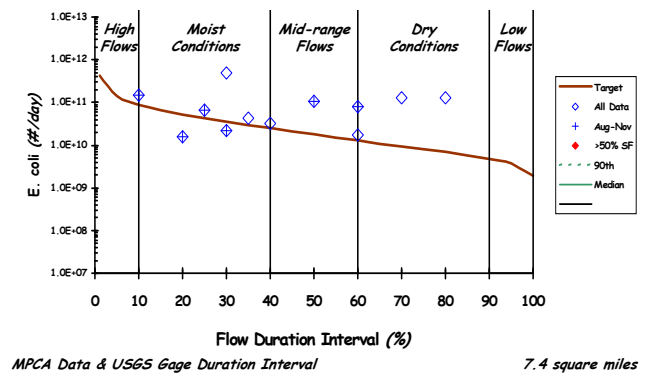
Site	Bovine <i>E. coli</i> Load		Non-Bovine <i>E. coli</i> Load	
	Percent of Samples Above <i>E. coli</i> Load Duration Curve	Sample Load Pattern (Flow dependent, Flow independent)	Percent of Samples Above <i>E. coli</i> Load Duration Curve	Sample Load Pattern (Flow dependent, Flow independent)
SC-2	25%	Dependent	75%	Dependent
SC-3	50%	Dependent	66%	Independent
SC-4	16%	Dependent	83%	Independent
SC-5	66%	Dependent	83%	Independent
SC-6	25%	Dependent	58%	Dependent
SC-7	25%	Dependent	25%	Dependent
GH-1	75%	Dependent	33%	Independent
NS-1	33%	Dependent	25%	Independent
HB-1	0%	NA	92%	Dependent

In this analysis the sum of the percent of samples above the threshold for bovine associated and non-bovine associated does not equal 100% for two reasons. First, in some samples (SC-7) the total *E. coli* load values were generally below threshold (Table 15), so it would be expected that partitioning of the load into bovine and non-bovine would lead to *E. coli* load values still below the threshold. Second in samples with loads greater than two-fold above the threshold and having both bovine and non-bovine fractions, separation into bovine and non-bovine load fractions may still leave both fractions above the threshold load value.

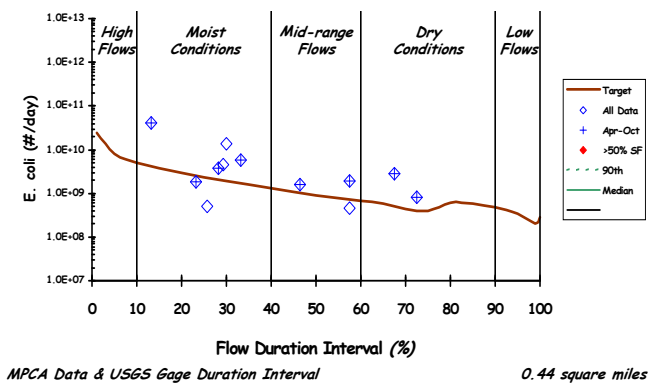
**SC-4 *E. coli* loads attributable to cattle using measured flows and predicted flow duration curves**



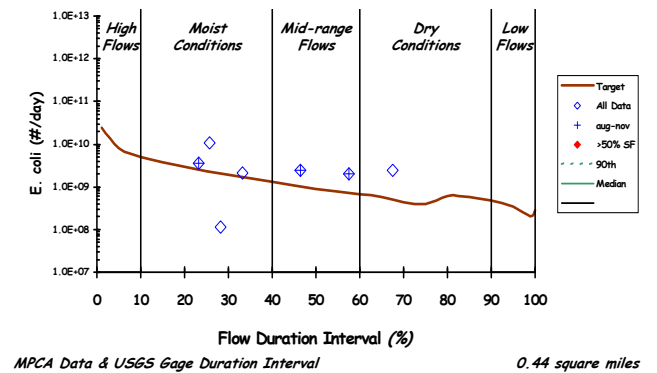
**SC-4 *E. coli* loads not attributable to cattle using measured flows and predicted flow duration curves**



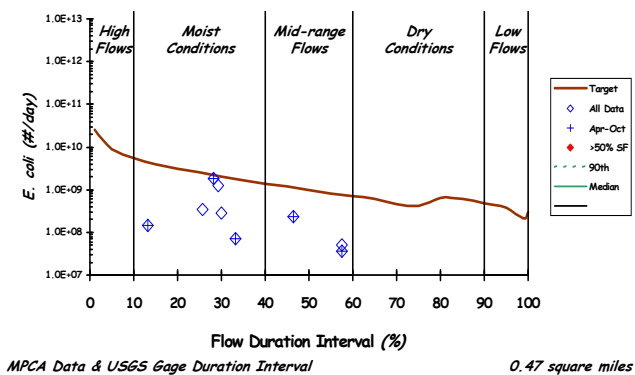
**GH-1 *E. coli* loads attributable to cattle using measured flows and predicted flow duration curves**



**GH-1 *E. coli* loads not attributable to cattle using measured flows and predicted flow duration curves**



**HB-1 *E. coli* loads attributable to cattle using predicted flows and predicted flow duration curves**



**HB-1 *E. coli* loads not attributable to cattle using predicted flows and predicted flow duration curves**

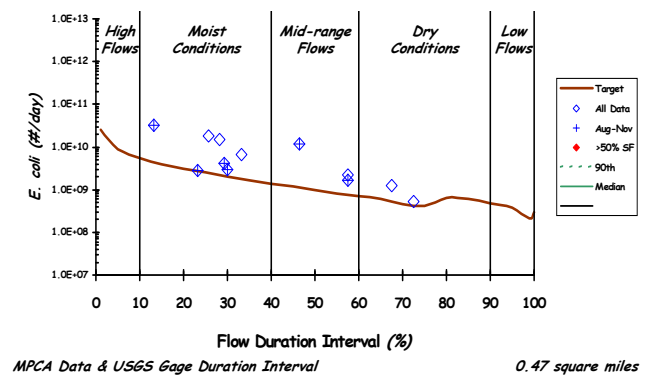


Figure 9. Examples of *E. coli* Load Patterns based on the separation of *E. coli* into bovine associated (left) and non-bovine associated (right).

Presentation of *E. coli* Load and Fecal Load Spatially

The above presentation of load allows one to examine the changes in load at specific locations in the watershed over time. An alternative more simplistic way to

examine is load is present load summarized over time and spatially. This presentation may be more compatible with TMDL development, as TMDLs generally do not consider changes in load with respect to time. The following method of presentation also allows one to locate the areas of highest loading along a water source and determine the relative impacts of load from tributaries. This type of graphical presentation may aid in decision making so that funding can be more effectively targeted.

In this section, the geometric mean of the flow measurements from sites SC2- through SC-7 were calculated across all twelve dates. The geometric means for flow at SC-1 and three tributaries (HB-1, GH-1, and NS-1) were calculated based on extrapolated flow calculations described above. The geometric means of the flows for the seven main creek sites are shown as a line graph relative to distance along the creek. In figure 10, drainage occurs from sites on the left to the right, with SC-7 representing the site closest to the headwaters and SC-1 representing the site closest to the mouth. The locations where the tributaries meet the main channel are indicated as bars at the approximate river distance. The relative flow of the tributaries can be compared to the flow of the main channel by the values on the y-axis. The volume of water in Stock Creek increases about 10 fold across the 6 mile stretch from SC-7 (2 CFS) to SC-1 (22 CFS). Neubert Springs and High Bluff tributary (HB) contributes about 16% and 11% of the flow respectively, where as Gun Hollow (GH) only contribute s about3% respectively to the flow at the next downstream Stock Creek location.

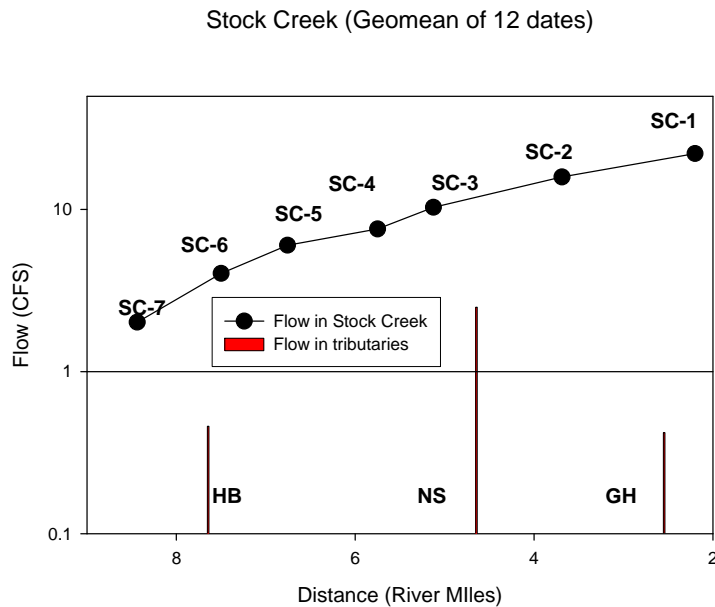


Figure 10. Flow versus distance at sample sites along Stock Creek and at three tributaries entering Stock Creek.

The *E. coli* load data described in the previous section was also summarized for each site by calculating the geometric mean for the twelve sample dates. In figure 11, the *E. coli* load attributable to each site along the main channel is plotted against the river mile distance. Because there is a regulatory threshold for *E. coli* concentration (126 CFU/100ml), an *E. coli* load limit for each site can be calculated. As one may expect, the *E. coli* load limit in the main channel (line graph) increases as the water flows downstream because of the increase in water volume. However, the *E. coli* load increases more rapidly as water travels downstream than the allowable load limit. The

area of the stream where with the *E. coli* load increases most rapidly is between SC-7 and SC-5. Although the *E. coli* load is below the limit at SC-7 by the time the water reaches SC-5, *E. coli* load is 3 fold greater than the *E. coli* load limit. In Stock Creek the *E. coli* may enter from sites along the main channel or from sites along the tributary banks. In this location of the watershed the only measured tributary with high amounts of *E. coli* was HB. This tributary contributes an equivalent of 16% of the water volume to and 15% of *E. coli* load to SC-6 (Figure 11). The other tributaries shown in Figure 2 also do not contribute significant *E. coli* loads to the main channel (10% by NS and 3% by GH).

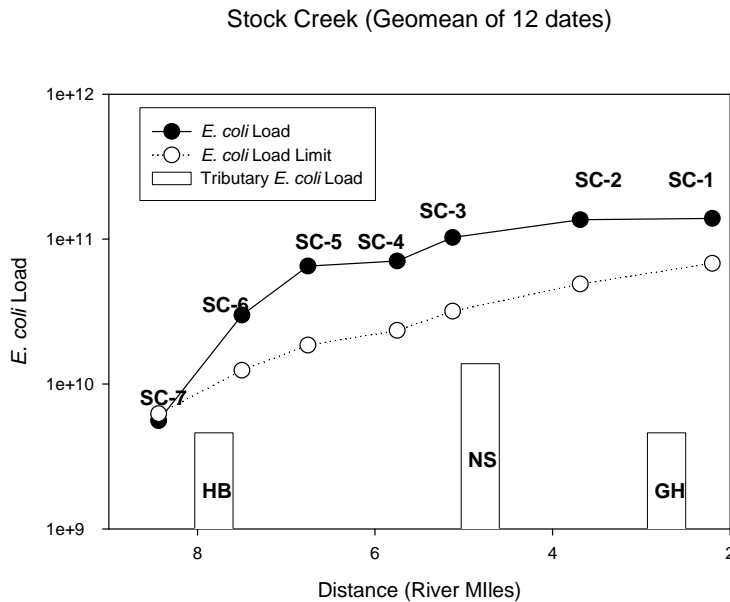


Figure 11. *E. coli* load profile of Stock Creek. ). Data points represent the geometric means of load calculations for each of the twelve sample dates across a one-year period. The locations where tributaries meet the main channel are shown on the x-axis and the pollutant load in indicated on the same y-axis as for the main channel.

Total fecal load and bovine fecal load was calculated based on the real-time PCR assays data, which had been converted to mg/L (Tables 6 and 7). The load was calculated as follows: Load (lb/day) = mg/L\* Flow (CFS)\* (5.38). To date there is no regulatory fecal concentration limit, so a fecal load threshold was not calculated. The fecal loads for each Stock Creek site and tributary were plotted against distance as shown in Figures 11 and 12 (Figure 13). The total fecal load pattern was similar to the *E. coli* load pattern (Figure 12) with about a 10 fold increase in fecal load at the downstream site (SC-1) compared to the upstream site (SC-7). As with *E. coli* load the fecal load increased most between the SC-7 and SC-5 sites and had a plateau between SC-5 and SC-4 sites. The tributaries added 5 to 17% of the fecal load to the downstream sites. In contrast, to the total fecal load pattern, the bovine fecal load dropped approximately 50% between SC-5 and SC-4. GH-1 and NS-1 contributed 18 and 17% of the bovine fecal load where as HB-1 contributed less than 2% of the bovine fecal load.



Stock Creek (Geomean of 12 dates)

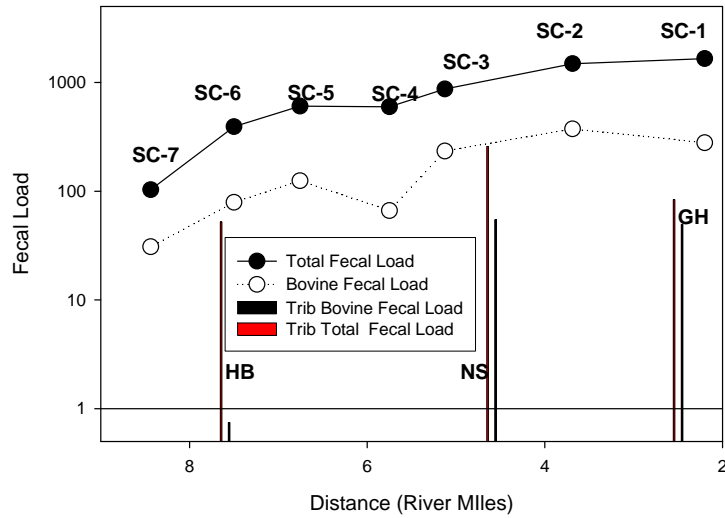


Figure 12. Fecal load profile based on *Bacteroides* assays (Right). Data points represent the geometric means of load calculations for each of the twelve sample dates across a one-year period.

### Section III.

**Based on the real-time PCR analysis, locations containing high levels of pathogens attributable to bovine fecal contamination were identified in the Stock Creek Watershed. However, several locations were identified that had high levels of fecal contamination not attributable to cattle. The objective of this section of the project was to identify the origin of fecal contamination at these sites (human versus horses versus wildlife). These sites were examined at high, medium and low water flows to determine whether the predominant type of source fecal contamination differs with type of flow in the watershed.**

*Bacteroides* 16S rRNA gene libraries were constructed for 20 water samples from a range of geographic sites along Stock creek and tributaries. The samples chosen for source identification via *Bacteroides* library analysis represent a range of *E.coli* concentrations (91 to 2419 CFU/100 ml) and flow conditions (dry to moist) (Table 17). Ten to twenty clones from each library were sequenced to determine the sources of fecal contamination for each water sample (Table 1). 58% of these sequences had a greater than 97% similarity to other sequences from known animal sources and therefore, could be assigned to an animal source with a high degree of confidence. **Therefore, across the watershed sequences identified with high confidence were predominantly assigned to either humans (63%) or cattle (33%) (Figure 13).** Another 4% of the sequences were attributed to horses or other animals.

When considered on a site-to-site basis, 12/13 sites (92%) had sequences of human fecal origin (14% to 63% of all sequences). In general the sites along the main branch of the creek contained mixtures of human- and cattle- associated *Bacteroides* sequences whereas, the samples from the sites at tributaries contained only human-associated or cattle-associated *Bacteroides* sequences. The GH-1 tributary site was the only site that contained sequences exclusively of cattle origin (GH-1), whereas 4 tributary sites only contained sequences of human origin (NS-1, GV-1, MB-1, HB-1).

Table 17. Source Identification in Stock Creek Watershed.

Site	Drainage (acres)	Date	<i>E. coli</i> (CFU/100ml)	Flow	% of sequences representing			# sequences
					Human (Fresh/septic)	Bovine	Other	
SC-2	9054	4/30/03	388	Moist	25 (25/0)	58	0	12
		8/26/03	1986	Mid	34 (28/6)	22	6	18
		10/09/03	326	Dry	29 (29/0)	41	0	17
		11/20/03	1120	Moist	22 (22/0)	28	6	18
SC-3	5557	11/20/03	980	Moist	78 (56/22)	22	0	18
SC-4	4755	10/30/03	2419	Dry	14 (7/7)	0	33	15
SC-5	2932	11/20/03	1986	Moist	33 (22/11)	28	0	18
SC-7	1035	4/30/03	91	Moist	15 (0/15)	15	0	13
		11/20/03	1120	Moist	45 (35/10)	30	0	20
GH-1	284	4/30/04	287	Moist	0	82	0	17
MM-1	639	4/30/03	118	Moist	27 (18/9)	0	18	11
		07/09/03	1120	Moist	52 (19/33)	0	0	16
SH-1	643	6/04/03	770	Mid	32 (8/24)	8	0	13
NS-1	1547	10/30/03	1300	Dry	39 (6/33)	0	0	0
GV-1	493	8/26/03	2419	Mid	40 (30/10)	0	0	10
MB-1	852	6/04/03	1203	Mid	35 (5/30)	0	0	20
HB-1	302	6/04/03	1414	Mid	61 (28/33)	0	0	20
		8/13/03	980	Moist	63 (63/0)	0	0	8
		10/09/03	344	Low	53 (33/20)	0	0	15
NM-1	999	11/20/03	1733	Moist	34 (28/6)	33	0	18

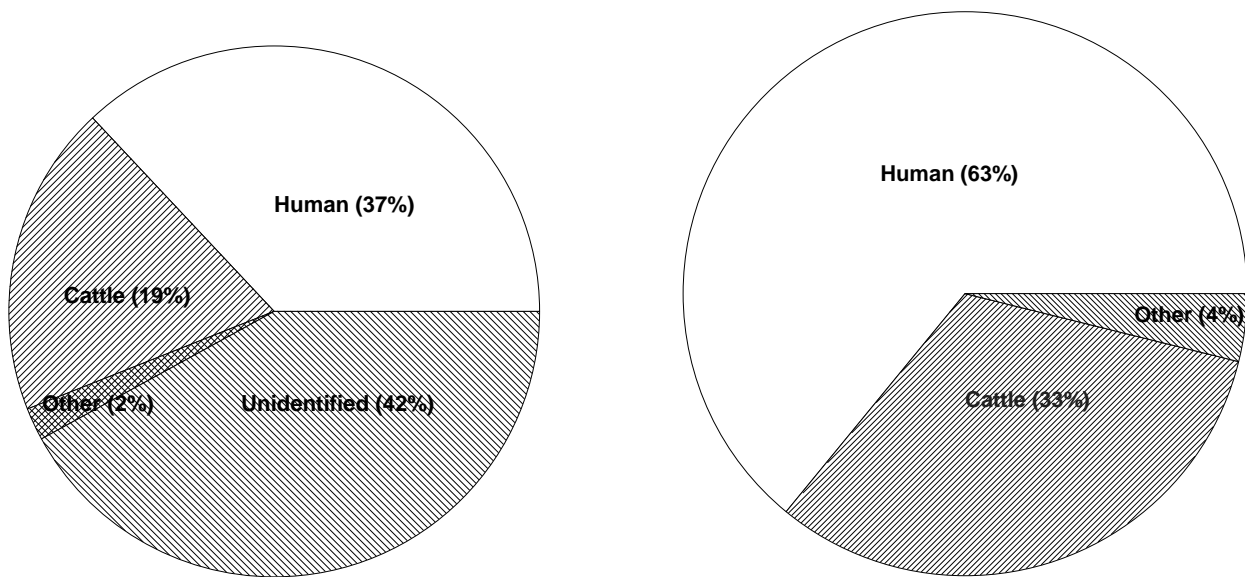


Figure 13. Distribution of *Bacteroides* sequences isolated from water samples collected in the Stock Creek Watershed. The pie chart on left shows the distribution of all sequence including sequences for which the source could not be conclusively identified (Unidentified group). The pie chart on the right shows the distribution of the sequences when only the sequences with a >97% sequences similarity to other reported sequences are considered. The other group contains horses, and other unknown animal hosts.