Impact of Surrounding Land Uses on Surface Water Quality

by

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Abstract

Source water protection is important to maintain public health by keeping harmful pathogens out of drinking waters. Non-point source pollution is often a major contributor of pollution to surface waters, and this form of pollution can be difficult to quantify. This study examined physical, chemical, and microbiological water quality parameters that may indicate pollution and may help to identify sources of pollution. These included measures of organic matter, particles, and indicator organisms (fecal coliforms and *E. coli*). The parameters were quantified in the West Boylston Brook in Massachusetts, which serves as a tributary to the Wachusett Reservoir and is part of the drinking water supply for the Metropolitan Boston area. Water quality was determined over four seasons at seven locations in the brook that were selected to isolate specific land uses. The water quality parameters were first analyzed for trends by site and by season. Then, a correlation analysis was performed to determine relationships among the water quality parameters. Lastly, ANOVA analyses were used to determine statistically significant variations in water quality along the tributary.

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1.0 Introduction

Much of the drinking water for the United States comes from surface water bodies such as lakes and rivers. For public health reasons, it is very important that drinking water sources or public water supplies are kept clean and free of pollution. Surface waters are vulnerable to pollution from their surrounding environment, and protection of surface waters is a complicated process that must often be done on a regional level.

Surface water bodies can be polluted from point source pollution as well as non-point source pollution. The latter of the two can be hard to locate, quantify, and/or regulate. Locating non-point sources of pollution to surface water bodies is challenging because the sources may be located within the entire watershed area. Runoff that travels over various land uses within a watershed is a major source of non-point source pollution. Through water quality monitoring and watershed land use surveys, public agencies work to locate and eliminate non-point sources of pollution to source waters.

The U.S. Environmental Protection Agency (EPA) oversees protection of water in the United States, which includes protection of surface waters and public water supplies. More locally, the Massachusetts Department of Conservation and Recreation (DCR) protects several water bodies in Massachusetts that serve as a public water supply for the Boston Metropolitan area. One of the reservoirs protected by the DCR is the Wachusett Reservoir located in central Massachusetts. This reservoir has had some non- point source pollution problems in several specific watershed areas or subbasins. The West Boylston Brook (WBB) watershed subbasin is one such area of the watershed that has had a history of pollution problems.

This study investigated the pollution problems in the WBB through physical, chemical, and biological water monitoring. Physical measurements included temperature, specific conductance, turbidity, and particle counts. Chemical measurements included pH, dissolved oxygen, and organic carbon (total organic carbon, dissolved organic carbon, and UV_{254} absorbance). Biological measurements included fecal coliforms and *E. coli*. Water quality parameters were measured at seven locations over a period of thirteen months in the brook to aid in determining the quality of the

water and the possible sources of pollution to the brook. Analysis of the data was conducted to determine contamination sources to the brook.

This study also evaluated the usefulness of various water quality parameters for source water protection. The collected data were analyzed to assess correlations between water quality parameters, and water quality differences among sampling sites and among seasons. Further analysis was carried out using a binary number system for several of the water quality measurements. Recommendations are provided for determining what land uses are polluting the surface water and for selecting which water quality parameters are most valuable for source water protection.

The next chapter describes the current literature on federal and state programs for monitoring and regulation, water quality parameters, and the affects of land uses on surface water quality. Chapter 3 discusses the methods used for collecting and analyzing water quality data as well as statistical analyses conducted on the data. Finally, the results of this study are presented in Chapter 4, and the recommendations on the usefulness of water quality indicators in monitoring surface waters are presented in Chapter 5.

2.0 Literature Review

Safe drinking water is essential for maintaining public health. Every effort should be made to achieve the highest quality drinking water possible. Protection of water supplies from contamination is the first step in providing clean drinking water. Source protection is one method of ensuring safe drinking water, and is used in conjunction with appropriate treatment and distribution procedures. For a source water protection program to be effective, pollution problems or risks within a watershed need to be identified. This is accomplished through watershed monitoring, which consists of water quality monitoring and land use surveys. This chapter provides background on source water protection strategies, regulations, land use impacts on water quality, and methods to identify pollution sources in a watershed.

2.1 Source Water Management

Source water is untreated water from streams, rivers, lakes, or underground aquifers which is used to supply private wells and public drinking water systems (EPA, 2005j). Source water management consists of protecting and treating source water to obtain adequate drinking water for a population. Source waters are protected from pollution as much as possible to reduce risks to public health. In addition, protecting a source water can be more economical than treating unprotected waters to obtain clean drinking water. Source water protection programs also protect valuable ecosystems for fish, other aquatic species, and wildlife, as well as preserve the natural environment for some recreational activities.

2.1.1 Surface Water Pollution

Surface water sources should be protected as much as possible from contamination by harmful pollutants. Potential pollutants include microorganisms, inorganic chemicals, organic chemicals, and radionuclides (EPA, 2005d). Completely protecting source water may not be possible because pollutants from the atmosphere can enter a surface water through precipitation, and contaminated ground water can introduce pollutants through recharge. However, limitations on land use around a surface water can reduce contamination of surface waters from the watershed itself.

Surface waters can be polluted by industrial and municipal discharges as well as altercations to the natural environment, which may cause runoff of pollutants. Both direct discharges and runoff can include human and animal waste. Human and animal feces may contain bacterial, viral, and protozoan pathogens as well as helminth parasites. Failure to provide adequate protection and effective treatment for drinking water can expose the community to the risk of intestinal and other infectious diseases.

Surface water pollution is classified into two major categories: point source pollution and non-point source pollution. Non-point source pollution, often in the form of runoff, comes from diffuse or scattered sources in the environment, while point source pollution comes from a defined outlet such as a pipe (EPA, 2005g). Non-point source pollution may be difficult to identify and control while point source pollution can be identified easily.

2.1.1.1 Point Source Pollution

Point source pollution, such as pipe discharges, industrial outflows, tributaries, or wastewater treatment plant outflows are relatively easy to define and regulate. The EPA regulates point source discharges throughout the United States with the National Pollution Discharge Elimination System (NPDES) permitting program. The NPDES program, which was introduced in 1972, requires point source dischargers to obtain permits from their state. This includes industrial and municipal dischargers, or any other facility that discharges wastewater to receiving water. The program greatly assists in the control of point source pollutants of anthropogenic origin. NPDES permits specify the allowable flow rate of a discharge and the maximum concentration of specific pollutants. The NPDES is an effluent based program which does not take into account the amount of pollutant that can safely be added to a specific water body without degrading that water. Therefore, the amount of pollutant that is allowed from a discharger is not dependant upon the size of the water body or the number of other dischargers to the water body.

Another tool to protect surface waters is the calculation of a Total Maximum Daily Load (TMDL) of a surface water. A TMDL is the total amount of a pollutant that a surface water can receive from all sources of pollution and still meet water quality standards. The Clean Water Act establishes the water quality standards for various

surface water uses (EPA, 2005k) The maximum load would be set differently if a water was used for drinking verses recreation (swimming and fishing), or for supporting aquatic life. A TMDL is a water body quality based regulation, and may be more stringent than a NPDES permit alone.

2.1.1.2 Non-point Source Pollution

Non-point source (NPS) pollution is typically caused by rainfall or snowmelt moving over or through the ground, picking up natural and human pollutants, and carrying those pollutants into surface waters. Pollutants include but are not limited to excess fertilizers, herbicides, and insecticides from agricultural lands and residential areas; oil, grease, and toxic chemicals from urban runoff and energy production; sediment from improperly managed construction sites, crop and forest lands, and eroding stream banks; salt from irrigation practices and acid drainage from abandoned mines; and bacteria and nutrients from livestock, pet wastes, and faulty septic systems (EPA, 2005l).

Non-point source pollution is a major problem for surface waters because it is often times difficult to identify the source of the pollution. Therefore, control of non-point sources of pollution is problematic. Often times, land use surveys and groundwater or surface water quality samples are the only ways of identifying where possible non-point sources may be located. NPS pollution is managed by states through watershed protection programs, which are discussed further in Section 2.2.2.

2.1.2 Drinking Water Regulations

The EPA sets and enforces drinking water regulations in the U.S. Under the Safe Drinking Water Act, the EPA advocates a "multiple barrier" approach to drinking water protection. The first part of the multiple barrier approach is source water protection. Source water protection includes assessing and protecting drinking water sources, protecting groundwater wells, and protecting surface water collection systems. The second part of the multiple barrier approach involves water treatment conducted by qualified operators. In addition, operators must ensure the integrity of distribution systems. Lastly, the multiple barrier approach requires water utilities to provide information to the public on the quality of their drinking water (EPA, 2005h).

The EPA drinking water standards apply to all public water supplies. Public water supplies provide water for human consumption through at least 15 service connections, or regularly serve at least 25 individuals. Public water systems include municipal water companies, homeowner associations, schools, businesses, campgrounds and shopping malls (EPA, 2005h).

There are two categories of drinking water standards: the national primary drinking water standards and the national secondary drinking water standards. The national primary drinking water standards are legally enforceable standards that apply to public water systems. Primary standards protect drinking water consumers by limiting the levels of specific contaminants that can adversely affect public health and are known or anticipated to occur in water. The national secondary drinking water standards are non-enforceable guidelines regarding contaminants that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water. EPA recommends secondary standards to water systems but does not require systems to comply (EPA, 2005h).

Regulated contaminants can be grouped into three categories: non-carcinogens (not including microbial contaminants) which cause adverse non-cancerous health effects, carcinogens, and microbial contaminants. The latter category includes protozoa, viruses, and bacteria that cause adverse health effects (EPA, 2005h). Drinking water regulations set by the EPA include maximum contaminant levels (MCLs) and treatment techniques. Maximum contaminant levels are set to minimize health risks while also considering the cost of treatment processes for removing contaminants. Microbial contaminant MCLs are set at zero because ingestion of one protozoan, virus, or bacterium could cause illness.

Any public water supply that obtains water from a surface water is regulated by the Surface Water Treatment Rule (SWTR) which was first promulgated by the EPA in 1989. The SWTR requires disinfection and filtration for all public water supplies that use surface water or a ground water that is under direct influence of surface water as a source (MDHHS, 2005). However, a filtration avoidance waiver can be granted to drinking water suppliers by the EPA if the surface water source meets specific quality requirements (MDHHS, 2005). The SWTR has also been expanded over the years with

the additions of the Long Term 1 Enhanced SWTR (LT1 ESWTR), and Long Term 2 Enhanced SWTR (LT2 ESWTR).

2.1.2.1 Filtration Waiver for Surface Water Supplies

Surface water supplies which meet strict criteria for quality and protection may apply for a filtration waiver under the SWTR. Systems which have a filtration waiver must continue to meet all MCL requirements, must disinfect the water, and must have the capability of redundant disinfection in case of microbial water contamination. In addition, the EPA also requires a watershed control program which encompasses many steps to minimize microbial contamination of the source water.

To be allowed filtration avoidance, the source water protection programs must characterize the watershed's hydrology, physical features, land use, source water quality and operational capabilities. Programs must also identify, monitor and control both human and natural activities that may deteriorate water quality. The watershed control program must also be able to control activities through land ownership or written agreements (EPA, 2005m).

To qualify for the SWTR filtration avoidance, source water must meet coliform bacteria and turbidity requirements. Ninety percent of the samples taken from the source water must have fewer than 100 total coliform bacteria per 100 ml, and fewer than 20 fecal coliform bacteria per 100 ml of source water (EPA, 1991). The turbidity of the source water prior to disinfection must be less than 5 ntu (EPA, 1991).

More current legislation from the LT1 and LT2 ESWTRs have set more stringent guidelines for water treatment. The LT2 ESWTR contains regulations set to protect against *Cryptosporidium*. The legislation calls for monitoring drinking waters for *Cryptosporidium* and many of the guidelines are based upon the levels of *E. coli* found in a source water.

2.2 Department of Conservation and Recreation

The Massachusetts Department of Conservation and Recreation (DCR), formerly known as the Metropolitan District Commission (MDC), manages and protects the drinking water supply for the communities in and around greater Boston. The Massachusetts Water Resources Authority (MWRA) treats and distributes the water to

communities in the Boston area. Greater Boston's drinking water system has a filtration waiver from the EPA because of high source water quality and the watershed protection provided by the DCR. Massachusetts has one of the largest source water systems in the world which serves nearly 2.5 million people in 46 different cities and towns throughout the commonwealth (DCR, 2005b).

2.2.1 DCR/MWRA Water System

The source water system for the greater Boston area is made up of three different watersheds including the Quabbin Reservoir, Wachusett Reservoir, and Ware River watersheds. The Quabbin Reservoir was built in the 1930's by damming the Swift River with the Windsor Dam, which flooded four Massachusetts towns located in the Swift River Valley. Hundreds of homes, businesses, a state highway, a railroad line and 34 cemeteries had to be moved out of the valley (DCR, 2005b). The reservoir has a volume of 412 billion gallons of water, covers 39 square miles, and is 18 miles long. The reservoir is fed by a 95,000 acre watershed (DCR, 2005b).

The Wachusett Reservoir was built in 1906 by damming the Nashua River in Clinton, MA. The reservoir, with a volume of 65 million gallons, has a surface area a 6.5 square miles and a 110 square mile watershed feeding into it from 12 different Massachusetts towns (DCR, 2005e).

The Ware River is used to provide additional water to the Quabbin Reservoir. From the months of October to June when the Ware River waters are high, a portion of the Ware River's water is diverted into the Quabbin tunnel, and piped into the reservoir. The Ware River is fed by a watershed consisting of approximately 62,000 acres (DCR, 2005b).

2.2.2 Watershed Protection

The Massachusetts Department of Conservation and Recreation protects source water quality through several means. The Watershed Protection Act (WsPA) was created by the Commonwealth of Massachusetts to protect drinking water sources, which includes the Quabbin Reservoir, Ware River, and Wachusett Reservoir, from pollution. With the WsPA, the DCR has the ability to restrict activities in the watersheds in order to reduce non-point source pollution. Some of the strategies used by the DCR include land

acquisition, use of a buffer zone along surface waters, limitations on impervious surfaces, and restrictions on the use and storage of hazardous materials within the watersheds.

One method of protecting water quality is the use of buffer zones. The DCR places various restrictions on land uses within 400 feet of any surface waters, flooding planes, or underground aquifers. The Watershed Protection Act defines two zones: the primary protection zone and the secondary protection zone. The primary protection zone covers all areas within 400 feet of reservoirs and 200 feet of surface waters or tributaries. The secondary zone covers all areas within 200 and 400 feet of tributaries and surface waters, on land within flood plains, over some aquifers, and within bordering vegetated wetlands (DCR, 2005a). Any alterations of land within the primary protection zone are prohibited, and there are many restrictions on land uses and activities within the secondary zone.

The DCR not only protects the source water system through land use restrictions, but also through land acquisition. The DCR has acquired significant acreage throughout the watersheds and continues to do so with the use of sophisticated computer models to identify the most sensitive land areas within the watersheds (DCR, 2005b).

Microbial and pathogenic contamination is partly controlled by restricting public access to the source waters and controlling some of the wildlife within the three watersheds. Human and animal presence on watershed lands increases the risk of pathogens contaminating the source waters. Public access is limited on DCR land, especially near any water intake structures. Swimming is not allowed in the reservoirs. Other human activities that may contribute to pathogen contamination include improper disposal of fecal waste from dogs, horses, or any other domestic animals on watershed land.

Some species of wildlife need to be controlled by the DCR because of their proximity to intake structures or because they can transmit harmful pathogens to humans. The animals that are targeted as threats to water quality are gulls, Canadian geese, ducks, beavers, muskrats and deer (DCR, 2005h). The goals of the wildlife control program are to minimize the presence of waterfowl at the water intakes to reduce high coliform counts at intake structures; to eliminate muskrat and beaver from sensitive water zones and zones near water intakes due to the harmful pathogens from the species; and to keep deer

populations at an appropriate number (DCR, 2005h). The main goal of the DCR's wildlife control program is to keep pathogens from the getting into the water intake, especially pathogens harmful to humans such as pathogens from beavers and a few other animal species.

2.3 Watershed Monitoring

In 1996, the Safe Drinking Water Act Amendments placed a renewed emphasis on source water protection (EPA, 2005j). States were given access to funding and required to develop Source Water Assessment Programs (SWAP). The goals of SWAPs are to identify potential threats to drinking water sources and initiate protection efforts. The four major elements of a SWAP are delineating or mapping source water assessment areas, conducting an inventory of potential sources of contamination, determining the susceptibility of the water supply to harmful contamination, and releasing results of the study to the public (EPA, 2005j).

2.3.1 Land Use Surveys

Land use surveys are useful for identifying possible sources of pollutants. Land use within a watershed is classified into several categories. An example of a few land use classifications are industrial or commercial, farmland, and residential. If there are known pollutants in a water body, a land use survey of the watershed area could be helpful in identifying where the pollutant or pollutants may have come from. Based on prior land use and water quality studies, certain land uses have been associated with specific contaminants.

Land use surveys also allow for a prediction of the risk of a pollutant entering the water. When land is acquired for source water protection, it is important that the funds to acquire land are used in the most beneficial fashion. A land use survey of a watershed would allow for the highest risk areas of land to be acquired.

Land use surveys are an important tool in water quality monitoring and source water protection, but a land use survey needs to be used in conjunction with water quality monitoring to be completely effective. Land use surveys may yield possible pollutant threats, but water quality then needs to be tested to isolate true sources of pollution.

2.3.2 Water Quality Monitoring

Water quality monitoring can be used to protect source waters by identifying pollutant levels and locations in a source water. Water quality monitoring is commonly done multiple times a year because water quality may change with season and with weather events. Water quality can be monitored by measuring physical, chemical, or biological characteristics of the water.

2.3.2.1 Physical Water Quality Monitoring

Physical measurements consist of measuring water temperature, flow, specific conductance, turbidity, and the condition of stream or lake banks. Physical characteristics are often related to chemical parameters. For example, eroding stream banks may be the cause of high suspended solids or low flow may be the cause of low dissolved oxygen content. Three measurable physical parameters that were measured in this study include temperature, specific conductance, and turbidity.

2.3.2.1.1 Temperature

The temperature of the water may affect both chemical and biological water characteristics. Rates of many biological and chemical processes vary with temperature. Biological water quality may vary with temperature due to varying species survival rates in different temperatures.

A chemical characteristic that varies with the temperature of the water is dissolved oxygen content. The saturation value of dissolved oxygen in water is inversely related to the temperature of the water. Therefore, in temperate climates, levels are typically higher in the winter and lower in the summer.

2.3.2.1.2 Specific Conductance

Specific conductance or conductivity of water is a measure of the ease with which an electrical current can pass through water. A high conductivity is a result of the presence of inorganic dissolved solids that carry a charge. Some examples of dissolved solids that are able to carry a charge are iron, calcium, chloride and sulfate. The specific conductance of a surface water can be affected by both natural and anthropogenic factors in the watershed.

The natural conductivity of a surface water body that has not been affected by human activities depends mainly on the geography of the area. The conductivity of surface water can vary depending upon the type of rock or soil that the water has come in contact with. Water that has come in contact with granite bedrock tends to have a low conductivity and water that has come in contact with clay soils tends to have a high conductivity.

Other natural variations in surface water conductivity can be caused by the type or amount of biological activity in a surface water (Copertino *et al.*, 1998). For example, a high rate of decay of organic matter by biological processes can affect the conductivity of the water. Degradation of plant matter increases the dissolved solids as well as the conductivity in the water. Therefore, seasonal changed in biological activity may have an effect on the seasonal trends of conductivity levels.

The specific conductance of surface water is also influenced by human activities within a watershed. Pollutants that enter a surface water through runoff may raise or lower the conductivity of a surface water. Organic compounds such as oil or alcohol lower the conductivity of water because they lack the ability to carry a charge. Areas with a high percentage of impervious surfaces, such as urban areas, can yield runoff containing oils that may lower the conductivity of a nearby surface water (Mehaffey *et al,.* 2005). However, Detenbeck *et al.* (1995) found that the majority of high concentrations of inorganic suspended solids occurred in the early spring in an urban wetland which raised the conductivity.

Other human activities in a watershed that may raise the conductivity of surface waters include agricultural and residential land uses. During snowmelt periods, it has been shown that surface waters surrounded by agricultural lands have a higher specific conductivity when compared to other land uses (Detenbeck *et al.*, 1995). A failing septic system near a surface water body could raise the conductivity of that surface water due to the presence of chloride, phosphate, and nitrate. Even a properly working septic system can affect the conductivity of nearby surface water. For example, New England area homes with septic systems to treat wastewater often times have wells to provide drinking water. Water coming out of a well from bedrock often has a low conductivity. This

water may eventually be discharged to shallow ground water or surface water via septic system, affecting the conductivity of the receiving water (Burns *et al.,* 2005).

2.3.2.1.3 Turbidity

Turbidity is a measure of the amount of suspended solids in a surface water. Suspended solids include soil particles, algae, and microbes (EPA, 2005n). These substances enter into a water body through non-point source pollution, such as soil erosion and urban runoff, and through processes within the water body, such as algal growth.

Turbidity levels in surface waters have been found to vary due to variations in precipitation and the percentage of impervious surface in a watershed. Long and Plummer (2004) found turbidity levels in a small stream to vary with changes in precipitation. Volk *et al.* (2002) found that turbidity levels in a stream could increase by as much as 300 fold during or following precipitation events.

High turbidity levels in surface waters have been linked to high percentages of impervious surfaces within a watershed caused by sediment loading from runoff and erosion. (Mehaffey *et al.,* 2005; Nelson and Booth, 2002) In contrast, Shoonover *et al.* (2005) found that during baseflow, turbidity concentrations were lower within watershed with higher percentages of impervious surfaces. Additionally, during typical storm events, urban watersheds seemed to have similar turbidity as rural watersheds. The differing relationships between turbidity levels and land use may be due to the specific watersheds included in each study. However, all of the studies were consistent in finding higher turbidity levels is surface water during periods with higher stream flow or precipitation levels.

2.3.2.2 Chemical Water Quality Monitoring

Chemical water quality parameters may be used to indicate sources of pollution or be linked to other physical or biological water quality parameters. Chemical monitoring in this study consisted of measuring pH, dissolved oxygen concentration, total and dissolved organic carbon levels, and UV_{254} absorbance.

2.3.2.2.1 pH

pH, which is a measure of the concentration of free H^+ ions, is affected by acid rain, surrounding rock formations, and certain wastewater discharges. Most fresh water aquatic species prefer a pH range of 6.5 to 8.0 (EPA, 2005n). Certain types of organisms prefer different ranges of pH; if the pH is high or low it will change the types of organisms found in a surface water. A low pH can also allow toxic elements and compounds to become mobile and available for uptake by organisms (EPA, 2005n).

2.3.2.2.2 Dissolved Oxygen

Dissolved oxygen (DO) enters a water body from the atmosphere and from oxygen producing plant life living in the water. The atmosphere is made of approximately 20 percent oxygen or 200,000 ppm; typical oxygen levels in surface waters are below 10 ppm (Department of Wildlife and Fisheries Sciences, 2005).

There are many factors that influence the amount of DO in a surface water. Oxygen is transferred from the atmosphere into moving waters (streams and rivers) at a higher rate than still waters, such as lakes. Water at a colder temperature also has a higher saturation level of dissolved oxygen than water at a warmer temperature. In addition, water at a lower altitude has a higher saturation level than water at a higher altitude. The turbidity of a surface water can also affect DO levels. High turbidity in surface water can reduce the level of DO by raising the water temperature and lowering photosynthesis due to the absorption of sunlight by suspended solids.

DO levels can provide information on the concentration of oxygen demanding pollutants that may be entering a surface water via point and non-point sources. Oxygen is consumed by microorganisms as they degrade organic matter in a water, which reduces the DO concentration. These oxygen demanding substances may arise from farmland runoff, urban runoff and septic systems. In particular, fertilizers, animal waste (livestock or wildlife), and human waste contribute to the oxygen demand.

2.3.2.2.3 Organic Carbon

Organic carbon is found naturally in surface water but may also be affected by human activities. The level of organic carbon in a surface water is related to various characteristics of a watershed including land use, seasonal temperatures, and the amount

of precipitation a watershed receives. Natural organic carbon originates from plant life in or around the surface water and from soil runoff. Organic carbon levels can be measured in terms of total organic carbon (TOC) or dissolved organic carbon (DOC).

TOC levels in surface water have been shown to be dependent on the amount of erosion entering a surface water. The type of soil that is eroding into a surface water will dictate how much the erosion will effect the TOC content. A study in the Rhode Island area found the top 1 cm of forest land soil contained 4.3% organic carbon and the top 1 cm of soil in cropland contained 0.87% organic carbon (Correll *et al.,* 2001). Correll *et al.* (2001) indicates that erosion from croplands will raise the TOC levels more than the same amount of eroded soil from forestlands.

Erosion, which affects the amounts of TOC, is affected by land use and levels of precipitation. The highest fluxes in TOC occur in cropland areas rather than upland forested areas (Correll *et al.,* 2001). A flux in TOC levels indicates the source or the TOC is erosion due to precipitation events. According to Correll *et al.* (2001), precipitation variations can account for 54-66% of the variation in annual TOC fluxes in a small single land use watershed, and TOC in surface waters near cropland and forested land can be 3 to 5 times higher in a wet year than a dry year.

Along with the levels of TOC, the levels of DOC have also been found to increase with increased precipitation (Volk *et al.,* 2002; Correll *et al.,* 2001). Correll *et al.* (2001) found that 21-43% of the variation in concentration of DOC can be linked to discharge in a mixed use watershed, and Volk *et al.* (2002) found that DOC concentration could increase by as much as 3 fold when discharge also increased by 3 fold in a small stream. Although, TOC in surface waters adjacent to cropland is strongly related to the amount of runoff discharged, but DOC concentrations were not correlated with the watershed discharge (Correll *et al.,* 2001).

Soil erosion is not the only contributor to organic carbon in surface waters. Correll *et al.* (2001) found TOC concentrations to be significantly higher from upland forest than from other watershed land uses during low flow periods. These levels indicate that near croplands the amount of TOC in a surface water is dependent upon the amount of eroded soil entering the surface water, but upland forested areas have high levels of TOC that are not completely dependent upon soil erosion.

Flow and stream characteristics can affect DOC levels. In wetland areas surface waters with continuous flow were found to have lower dissolved organic carbon than wetlands without continuous flow (Detenbeck et al., 1995). In an upland forested watershed, watershed discharge was linked to the concentration of TOC present as DOC, and TOC concentrations were not linked to watershed discharge (Correll *et al.,* 2001).

There were some predominant trends between seasonal changes and the amount of organic carbon in a surface water. Correll *et al.* (2001) found a strong correlation between TOC and mean temperature for the summer and winter season; with higher temperatures in both seasons there were higher TOC concentrations (Correll *et al.,* 2001). In addition, TOC concentrations were found to be higher from upland forest areas than croplands in the fall season (Correll *et al.,* 2001). TOC concentrations were also found to be lower during snowmelt periods than during other spring sampling periods (Detenbeck *et al.,* 1995; Correll *et al.,* 2001), and DOC was higher in surface waters surrounded by agriculture than surface waters surrounded by any other type of land uses including urban, residential, and forest land during snowmelt periods (Detenbeck *et al.,* 1995).

2.3.2.2.4 Measure of Organic Matter with UV_{254}

Some dissolved organic compounds in water absorb ultraviolet light. Therefore, measurement of ultraviolet light absorption at a wavelength of 254 nanometers (UV_{254}) is a surrogate for organic matter concentration. For example, *Volk et al.* (2002) found that levels of UV_{254} increase during or after precipitation events in streams and fluctuate in correlation with dissolved organic carbon levels. However, UV_{254} had a higher level of variation than DOC (Volk *et al.,* 2002).

2.3.3 Microbiological Water Quality Monitoring

Microbiological water quality monitoring consists of measuring the concentration of microorganisms in a water body. Microorganisms of interest in surface water sources include bacteria, viruses, protozoa, and indicator organisms. Indicator organisms are used to indicate the presence of other potentially harmful pathogens. Two microorganisms that are commonly used for surface water quality testing are fecal

coliforms and *E. coli.* The presence of fecal coliforms and *E. coli* indicates contamination of a surface water from human or animal waste.

2.2.3.1 Fecal Coliforms and *E. coli* As Indicator Organisms

An indicator organism is an organism that can provide information about the health of a water body through the organism's presence, condition, or numbers (EPA, 2005b). For surface waters, total colifoms, fecal coliforms and *E. coli* are used to indicate the possible presence of harmful pathogens derived from human or animal waste. Some harmful pathogens of concern in source waters include *Cryptosporidium*, *Giardia*, and *E. coli* O157: H7 (not all *E. coli* are harmful).

An ideal indicator organism should be non-pathogenic, rapidly detected, easily enumerated, and have survival characteristics that are similar to any harmful pathogens of concern. For assessing public health risk, indicator organisms include fecal colifoms and *E coli,* which are normally found in the intestines and feces of humans, livestock, and wildlife. These organisms are relatively easy to detect and the enumeration process is relatively inexpensive (Meays *et al.*, 2004).

There are two problems with using indicator organisms. First, it is difficult to identify the source of the contamination. Second, it is not possible to know if bacterial contamination found in a source water indicates the presence of pathogens harmful to humans. If fecal coliforms are found in source water, it may be difficult to identify whether the contamination is coming from wildlife, farmland, or septic systems. It is also not possible to know from just testing for fecal coliforms if there are harmful pathogens present at all. The fecal material must come from an infected animal or human in order for harmful pathogens to be present.

There have been many links between different land uses and the amount of total coliforms, fecal coliforms, and *E. coli* found in nearby surface waters. High fecal coliform counts have been positively related to urban development, agriculture, and the amount of erodible soils (Mehaffey *et al.,* 2005). High coliform counts are not only dependent on the total area of a particular land use within a watershed, but also on the amount of precipitation that the watershed recieved in previous days (Mehaffey *et al.,* 2005; Stukel *et al.,* 1990).

Urban land use and agricultural land use seem to yield the highest concentrations of fecal coliforms in nearby surface waters (Mallin *et al.,* 2000; Mehaffey *et al*., 2005; Shoonover *et al.,* 2005). Agricultural land use yields high levels of fecal coliforms depending upon the amount of agricultural debris that can erode into surface waters. A high percentage of agriculture on very steep slopes adjacent to a surface water yields very high counts of fecal coliforms (Mehaffey *et al*., 2005). Vegetative strips or buffer zones have been found to reduce nutrient loadings to surface waters from agricultural land use, but fecal coliform counts were not reduced (Fajardo *et al.,* 2001).

In urban watersheds, a strong correlation exists between the percent impervious surface in the watershed and mean fecal coliform levels in suface waters (Shoonover *et al,.* 2005; Young and Thackston, 1999). Mallin *et al.* (2000) also found a similarly strong correlation between population density and mean fecal coliform levels in surface waters. Fecal coliforms are consistently higher during both base flow and high flow periods in urban watersheds compared to other land uses (Shoonover *et al,.* 2005).

The number of domestic animals in an urban watershed may be another cause of elevated fecal coliform levels (Mallin *et al.,* 2000; Young and Thackston, 1999). Young and Thackston (1999) found that high levels of fecal coliforms and *E. coli* in urban watersheds were the result of animal sources rather than human. They also found that fecal coliform counts from septic systems in surface waters of an urban watershed were negligible in comparison to other sources of fecal coliforms.

Fecal coliforms levels can be affected by various physical and chemical parameters of a surface water. Total coliform, fecal coliform and *E. coli* levels may be lower in surface waters with harsh living conditions for the organisms. The die off rate for the organisms can be accelerated, thus yielding possible lowered concentrations by cold temperatures in the winter or extreme pH levels. In an urban watershed, fecal coliform levels were much higher in the summer than in the winter (Young and Thackston, 1999). Turbidity is another surface water characteristic that may affect the levels of coliforms. Mallin *et al,* (2000) found that coliforms have a much longer survival rate when in association with suspended solids. High levels of fecal coliforms were correlated with high levels of suspended solids in the Chesapeake Bay.

2.3.3.2 Microbial Source Tracking

Microbial source tracking (MST) is a way of identifying the source of microorganisms in a watershed. By determining the pollutant source, an assessment can be made on the risk of pathogen contamination. There are many different methods of microbial source tracking including comparison of fecal coliform concentrations to fecal streptococci levels, identifying host specific organisms, genotypic analyses, antibiotic resistance analyses, and chemical identification. The following sections discuss the first two MST tools.

2.3.3.3 FC/FS Ratios

One form of microbial source tracking consists of determining the ratio of fecal coliforms verses fecal streptococci, or the FC/FS ratio. Initially it was thought that a ratio of > 4.0 would indicate human pollution and < 0.7 would indicate non-human pollution because observations had been made that human feces contained higher fecal coliform counts and animal feces contains higher levels of fecal streptococci (Scott *et al.*, 2002). This method of microbial source tacking has been proven unreliable due to varying survival rates of fecal streptococci (Scott *et al.*, 2002).

2.3.3.4 Host Specific Organisms

Another form of microbial source tracking is finding bacteria that are present only in the intestines of specific species or host specific organisms. A valuable host specific organism for source water protection is an organism that is either human specific or animal specific and that can survive in the environment for a certain period of time. Three host specific organisms that may be used for source water protection are strains of *Bifidobacteria, Rhodococcus coprophilus,* and *coliphages.*

2.3.3.4.1 *Bifidobacteria*

Strains of *Bifidobacteria* can be used as an indicator of human contamination in a source water. These bacteria are a major part of the human intestine, are rarely found in animals (Scott *et al.,* 2002), and certain strains of *Bifidobacteria* are only found in humans (Gavini *et al.*, 1991). *Bifidobacteria* have not been found in unpolluted environments such as springs, uncontaminated soil, or garden compost (Evison *et al.*,

1975). Differentiation is done to determine human strains on the basis of sorbitol fermentation (Mara *et al.*, 1983). The two more prevalent human strains include *Bifidobacterium adolescentis* and *B. breve* (Mara *et al.*, 1983).

2.3.3.4.2 *Rhodococus coprophillus*

Strains of *Rhodococcus coprophilus* can be used as indicators of animal contamination, specifically livestock. *Rhodococcus coprophilus* is found in the feces of farm animals (cattle, chickens, ducks, geese, horses, pigs, sheep, and turkeys) and in waters polluted by fecal material from these sources (Finlay *et al.*, 1998). Two successful source tracking studies have shown that the presence *Rhodococcus coprophilus* can indicate grazing animal fecal contamination (Long *et al.*, 2003; Long *et al.* 2004).

2.3.3.4.3 *Coliphages*

A group of *coliphages*, F+RNA *coliphages,* have been the most widely researched coliphages for microbial source tracking (MST) applications. F+RNA *coliphages* are a group of icosahedral phages which are morphologically similar to several human enteric virus groups, and thus have been proposed as indicators of enteric viruses (Sinton *et al.,* 1998). Furthermore, it was demonstrated through serotyping and genotyping that the majority of F+DNA coliphage isolate M13 were from domestic wastewater sources, making it a possible human-specific viral indicator (Long *et al.,* 2005).

3.0 Methods

The main goal of this project was to assess water quality in the West Boylston Brook as well as to determine what water quality parameters should be used to indicate pollution. Seven sampling locations were chosen in the West Boylston Brook to distinguish several different land uses in the watershed sub-basin. This chapter provides information on the sampling protocol. Second, this chapter discusses the analytical procedures used for measuring each water quality parameter. Lastly, the chapter details how statistical analyses were performed on the water quality parameter data.

3.1 Experimental Design

Water quality can be assessed through physical, chemical, and microbiological characteristics. Water quality can vary due to human or animal pollution, climate, and watershed characteristics. Sources of pollution from human activities can be determined through measurement and analysis of several water quality parameters. This type of watershed monitoring is useful for development of source water protection programs.

The West Boylston Brook (WBB) watershed is a sub-basin watershed located in the Wachusett Reservoir watershed in Central Massachusetts. The Wachusett Reservoir is one of the reservoirs which provides drinking water to Boston and the surrounding communities. The West Boylston Brook sub-basin watershed is one of the most developed and most polluted sub-basin watersheds in the Wachusett Reservoir watershed. The WBB has historically had high levels of fecal coliforms and high turbidity levels.

3.1.1 Sampling Locations

Seven sampling locations were chosen along the WBB to determine possible sources of pollution to the brook. The first site, Gate 25, is the sampling site used by the Department of Conservation and Recreation (DCR) near the confluence of the brook and reservoir. This site as well as six other upstream locations were chosen based on surrounding land uses and isolation of potential pollution sources.

The six upstream sampling sites were lettered A thru F. Gate 25, Site A, Site B, Site D and Site E were all located in the main channel of the WBB. Sites C and F were located in two other natural channels that feed into the main channel of the WBB. All of

the sampling locations were no more than approximately a half mile upstream of the reservoir. Detailed descriptions of the site locations and characteristics are provided in the Results Chapter.

3.1.2 Sampling Dates

Sampling was done approximately once a month from February 2005 to February 2006. A total of ten sampling events were completed over these 13 months. Sampling events included both wet and dry weather conditions. Two of the sampling events were completed during rain events (in May 2005 and October 2005). Sampling was done during all four seasons. There were 3 winter sampling events between December 21st and March 21st; 2 spring sampling events between March 21st and June 21st; 3 summer sampling events between June 21st and September 21st; and 2 fall sampling events between September 21st and December 21st. The exact sampling dates and the precipitation conditions for those sampling events are provided in the Results Chapter.

3.1.3 Sampling Protocol

Sampling was done at the same time of day (mid morning) each sampling trip. Samples were taken from the most downstream site first and continued up to the most upstream site. Sampling in this manner ensured that downstream water quality was not altered due to disturbances of upstream sites when sampling.

The first step in the sampling process was to note flow conditions, any changes in surrounding wildlife or human activity, the location that the sample was taken from, and the time the sample was taken. At Gate 25, the flow was further characterized by recording the stage at a stage station located at the site. Next, the water sample was collected directly from the stream.

Samples were collected using either a 1 liter or a 4 liter sterilized bottle. If the flow was deep enough, samples were taken directly with a 4 liter sterilized high density polypropylene (HDPP), Nalgene, wide mouth screw capped bottle (Nalge Nunc International, Rochester, New York). If the flow was not deep enough, the sample was collected with a 1 liter sterilized high density polypropylene (HDPP), Nalgene, wide mouth screw capped bottle (Nalge Nunc International, Rochester, New York) and then poured into the 4 liter bottle multiple times to fill the larger bottle. The sampler wore

rubber gloves sprayed with ethanol. Holding the bottom of the sample bottle, the mouth of the bottle was faced directly upstream and submerged into the middle of the stream to mid-depth or at the water surface if the flow was too shallow to obtain mid-depth samples. While taking the water samples, it was important not to disturb anything upstream of the sampling bottle or any of the sediments in the area from which the sample was taken. The full 4 liter sample bottles were then taken back to the car and placed in a cooler for transport to the laboratory.

The last step in sampling was to measure the specific conductance, dissolved oxygen, and temperature of the water with field instruments and in accordance with Standard Methods (APHA *et al.,* 1998; see Table 1). These parameters were measured with field probes. The specific conductance and dissolved oxygen content of the stream water were measured with a YSI 30 salinity conductivity meter (Yellow Springs, YSI, OH) and a YSI 95 DO meter (YSI, Yellow Springs, OH), respectively. Both meters also provided a temperature reading. The probes on each device were placed in the center of the stream at approximately half of the total depth. The probes were placed in undisturbed stream water, 1-2 meters upstream of where the sample water was collected. Measurements were recorded once stable readings were obtained.

Table 1: Field Measurements

Parameter	Instrument	Standard Method Number
Temperature	YSI 95 DO meter, YSI, Yellow Springs, OH	2550
Dissolved Oxygen	YSI 95 DO meter, YSI, Yellow Springs, OH	4500-O
Conductivity	YSI 30 salinity conductivity meter, Yellow Springs, YSI, OH	2510

3.1.4 Transporting and Splitting Samples

The samples were transported to the laboratory in the 4 liter HDPP bottles. The bottles were placed in coolers with ice packs and a thermometer, which was used to verify transportation temperatures. Samples were split at the DCR laboratory in West Boylston, MA directly after the last sample had been collected. The 4 liter bottles were inverted 20 times and this water was used to fill several smaller sterilized bottles aseptically. For the analyses conducted at WPI, a one liter HDPP bottle was filled with sample water and placed back in the cooler with ice and a thermometer to be brought to the laboratory at WPI. Remaining sample water was used for analyses at the University of Massachusetts laboratory.

3.2 Laboratory Analytical Procedures

The water characteristics that were measured at Worcester Polytechnic Institute in the Environmental Engineering Laboratory are shown in Table 2. They include turbidity, pH, UV_{254}, particle counts, fecal coliforms, *E. coli*, total organic carbon and dissolved organic carbon. The samples were split upon arrival to the Worcester Polytechnic Institute Water Laboratory. Each bottle was thoroughly mixed, and sample water from each site was poured aseptically from the 1 liter bottle into a 250 ml Nalgene, wide mouth screw capped bottles (Nalge Nunc International, Rochester, New York). The sample water in the 250 ml bottle was used for pH, turbidity, UV_{254}, TOC, DOC, and particle counts. Sample water remaining in the 1 L bottle was used for fecal coliforms and *E. coli*. Aseptic conditions were maintained for these latter two analyses.

Table 2: Laboratory Tests

Parameter	Instrument	Standard Method Number
Turbidity	2100N, Hach Company, Loveland, CO	2130
pH	AB15, Fisher Scientific, Pittsburgh, PA	4500-H^+
UV_{254}	Cary 50, Varian, Palo Alto, CA	5910
Particle Count	PC 2400 PS, Chemtrac Systems Inc., Norcross, GA	2560
Dissolved Organic Carbon	TOC-5000A, Shimadzu, Colombia, Maryland	5510
Total Organic Carbon	TOC-5000A, Shimadzu	5510
Fecal Coliforms	not applicable	9222 D
E. coli	not applicable	9222 G

3.2.1 Turbidity

The turbidity was measured using a Hach Model 2100N Laboratory Turbidimeter, (Loveland, CO) and in accordance with Standard Method 2130 (APHA *et al.,* 1998). Sample water from each site was poured into a clean, oiled turbidity vial after the sample bottle had been inverted several times. The turbidity vial was filled to the white line, gently inverted several times, and placed into the turbidimeter (making sure to align the white arrow on the sample cell to the white line on the turbidimeter). A measurement was obtained by waiting 15 seconds, watching the digital readout on the turbidimeter for 30 seconds and determinining an average reading. Two replicate measurements were recorded for each sample. The turbidimeter was calibrated every 4 months with Stabl Cal Calibration standards of less than 0.1, 20, 200, 1000, and 4000 ntu (Hach Calibration Standards Catalog Number 226621-05).

3.2.2 pH

The pH of the sample water was measured with a Fisher Scientific AB15 pH meter (Pittsburgh, PA) in accordance with Standard Method 4500-H^+(APHA *et al.,* 1998). Sample water was poured into a small clean beaker from the 250 ml sample bottle after inverting several times. The pH meter was calibrated before use with 4, 7, and 10 pH buffers. The pH probe was then placed in the sample and the value read from the digital readout of the calibrated pH meter.

3.2.3 UV_{254}

The absorbance of UV light at a wavelength of 254 nm was measured with a Varian Cary 50 Spectrophotometer (Palo Alto, CA) in accordance with Standard Method 5910 (APHA *et al.,* 1998). Sample water from each site was prepared by filtering through a glass fiber filter (Whatman GF/F filter with 0.7 µm retention). First, the filters were pre-washed with 20-30 ml of E-pure water. Then the sample was filtered. The first 5-10 ml of the sample was discarded, and next 10 ml was filtered into an acid washed 40 ml glass vial. From the 40 ml glass vial, the sample water was transferred into a Varian 10 mm, rectangular stoppered quartz spectrophotometer cell (Palo Alto, CA). The spectrophotometer cell was pre-washed with E-pure water and wiped with a Kimwipe. The spectrophotometer cell was then filled with filtered sample water and inserted into the spectrophotometer which yielded a digital readout. Duplicate measurements were recorded for each sample (emptying and cleaning the spectrophotometer cell between readings). Two samples of E-pure water were measured to serve as zero readings. The average zero reading was subtracted from each sample reading to provide the true absorbance.

3.2.4 Particle Counts

Particle counts were measured using a Chemtrac Systems PC 2400 PS Particle Counter with Grabbit 311 Software (Chemtrac Systems Inc, Norcross, GA). The software was set up to purge with 25 ml of sample and then count particles in two subsequent 50 ml volumes of sample. The particle size intervals that the counter was set up to measure were: 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-15, 15-20, 20-30, 30-40,

40-50, 50-75, 75-100, and 100 µm and greater. E-pure water (125 ml) was run through the instrument in between samples to ensure no carry-over.

The particle counts were done by first testing the port between the computer and the particle counter. The software program settings were then downloaded to the particle counter from the computer. Next, the flow rate of the particle counter was adjusted to 100 ml per minute. This was done using a graduated cylinder and stopwatch, and adjusting the flow rate as needed.

To analyze the samples, the correct "tag name" was selected for the sample, and the counter started by pressing the start button. After each sample, the data was saved. An E-pure sample was run between each sample to assure complete flushing of the particle counter. After all samples were analyzed, the data were uploaded back to the computer and saved in MS Excel 4.0.

3.2.5 Fecal Coliforms

Fecal coliforms were measured in accordance with Standard Method 9222D, the membrane filtration technique (APHA *et al.,* 1998). This method involves filtering a volume of the sample, placing the filter on a Petri dish with nutrient media and allowing time for bacterial colonies to grow. Sterile conditions were maintained throughout this analysis. Ideally, Petri dishes have 20-60 colonies per plate. To achieve this count range, three or four different volumes of water were filtered depending upon previous levels of fecal coliforms and weather conditions on the particular sampling date.

3.2.5.1 m-FC Agar

Fecal coliform colonies are incubated on Petri dishes with m-FC agar (Difco # 267720, Sparks, MD). To prepare the agar, 52 grams of the agar was suspended in 1 liter of E-pure water in a sterile Erlenmeyer flask. The agar was heated to boiling and allowed to boil for 1 minute with stirring. Then 10 ml of a 1% rosolic acid (prepared by dissolving 0.1 g of stock rosalic acid in 10 ml of 0.2 N NaOH) was then added to the agar. The agar was boiled for another minute. The agar was then cooled in a water bath to 47 degrees Celsius.

Once the agar was cooled to 47ºC, 5 to 6 ml of the agar was dispensed into 50 x 9 mm Petri dishes and allowed to solidify. The Petri dishes containing solidified agar were

stored upside down at 4ºC in sealed plastic bags for a maximum of two weeks. The pH of the agar was checked on two plates to assure that it was 7.4 ± 0.2. The night before using the plates, they were warmed in the 35ºC incubator.

3.2.5.2 Buffered Water

Buffered water is a solution that neither prohibits nor enhances growth of microorganisms. The buffered water is used for dilution of sample water as well as washing the filter apparatus. Buffered water is made by diluting 1.25 ml of stock phosphate buffer and 5 ml of of stock magnesium chloride up to 1 L of E-pure water. The stock magnesium chloride was made by dissolving 20.275 g of $MgCl_2 \cdot 6H_2O$ to a total volume of 250 ml of E-pure and the stock phosphate buffer was made by suspending 8.5 g of KH_2PO_4 up to 125 ml of E-pure.

3.2.5.3 Filtering

A filtration apparatus was assembled including a vacuum pump, filtration manifold, collection flask, and 47 mm filter funnels. First, flamed tweezers were used to transfer a sterile 0.45 µm gridded membrane filter (Millipore Corp., Billerica, MA) onto the sterile filter tower apparatus (with the gridded side facing upwards). The filter towers were pre-filled with 10 ml of buffered water for any water sample volumes that were below 10 ml. The water sample was then taken directly from the sample bottle after inverting it three times and transferred into the filter tower. The vacuum pump was turned on and the tower was washed with buffered water from a squeeze bottle. Once the water was filtered through the membrane, the vacuum pump was turned off. The filter paper was lifted with sterile tweezers and placed into a labeled Petri dish on top of the m-FC agar, ensuring that the paper was in contact with the agar media. The filter tower was then washed with buffered water and the filtration steps were repeated.

Three replicate Petri dishes were prepared for each volume filtered, and 3 to 4 volumes were filtered for each site. This yielded 9 to 12 plates for each sampling site. After the 3 replicate plates for a particular volume were prepared, the Petri dishes were wrapped and sealed in plastic bags and placed upside-down in a water bath at 44.5 ± 0.2 ºC for 24 ± 2 hours.

After 24 hours the Petri dishes were taken out of the water bath and the fecal coliforms and background colonies were counted at 10-15 times magnification in accordance with Standard Methods. A fecal coliform colony was specified as an individual, blue-green, circular, symmetrical growth with a metallic shine. A background colony was any other form of individual, peach or cream, circular, symmetrical, growth with a metallic shine.

3.2.6 *E. coli*

E. coli were enumerated in accordance with Standard Method #9222 G (APHA *et al.,* 1998). This method utilizes the Petri dishes prepared for fecal coliform analysis as described in the following two sections.

3.2.6.1 Nutrient Agar with MUG

Petri dishes for *E. coli* enumeration were made with nutrient agar with MUG (Difico #211825, Sparks, MD). First, 16.17 grams of the agar was measured and put into 700 ml of E-pure water in a beaker. The solution was heated to dissolve. The solution was then transferred to a 1 L screw top Erlenmeyer flask and autoclaved for 30 minutes at 121 degrees Celsius. After autoclaving, the solution was then allowed to cool to 47ºC in a water bath before the agar was to be plated. Then, 5 to 6 ml of the agar was aseptically plated into Petri dishes, allowed to solidify, and stored upside-down at 4ºC in the dark for no more than one week. The pH of the agar was tested using litmus paper to ensure a pH of 6.8 ± 0.2. The night before using the plates, the plates were warmed in the 35ºC incubator.

3.2.6.2 *E. coli* Enumeration

Once the fecal coliforms were counted and recorded, the filter membrane was aseptically transferred from the Petri dishes containing m-FC to identically labeled Petri dishes containing nutrient agar with MUG. The transfer was done by lifting the membrane on the m-FC plate by the edge with flamed forceps and lowering the paper into the new Petri dish on top of the nutrient agar with MUG, making sure no air bubbles were under the filter paper. The location of each of the fecal coliforms was then marked on the cover of each Petri dish. This was done so that the fecal coliform and background

colonies could be differentiated when counting the *E. coli* colonies, as all colonies take on a beige appearance after incubation on nutrient agar with MUG.

Following the membrane transfer, the Petri-dishes were placed upside-down into a 35ºC incubator for 4 hours ± 20 minutes. The Petri dishes were then taken out of the incubator to count the *E. coli* colonies. An ultraviolet light (Linterna Spectroline, Spectronics Corp., Westbury, New York) was held over the Petri dish. The number of colonies that fluoresced under the light were counted and recorded as *E. coli*. Once all of the counts were recorded, the plates were autoclaved in a biohazard bag and disposed of.

3.2.7 Positive and Negative Controls for Fecal Coliforms and *E. coli*

In addition to the samples, negative and positive controls were also filtered. There were seven pre-negative controls. These consisted of 10 ml of buffered water filtered through a membrane in each filter tower before the water samples were filtered. For post-negative controls, 10 ml of buffered water was filtered through a membrane in each filter tower after all the water samples had been filtered.

E. coli was used for the positive control in order to test the ability of the m-FC media to grow fecal coliforms and *E.coli*. *E. coli* was grown overnight in a 100 ml shaker flask with nutrient broth. Tryptic soy broth (Bacto # 211825, Sparks, MD) was prepared by weighing 3 grams of tryptic soy broth powder and dissolving it into 100 ml of E-pure water. The nutrient broth was split into two shaker flasks and autoclaved. After cooling, the shaker flasks were inoculated with *E. coli* from a frozen stock. The flasks were then incubated at 35ºC on a rotating platform at 100 revolutions per minute. Three dilutions were then filtered, incubated, and counted in the same manner as the water samples for both fecal coliforms and *E. coli*. The filtered volumes were 1 ml of 10^{-8} dilution, 0.1 ml of 10^{-6} dilution, and 1 ml of 10^{-6} dilution.

3.2.8 Total and Dissolved Organic Carbon

Total and dissolved organic carbon levels were measured in accordance with Standard Method 5510 (APHA *et al.,* 1998). All of the glassware used for the total and dissolved organic carbon analyses was washed by means of a 20% sulfuric acid bath for a minimum of one hour and rinsed 3 times with E-pure water. On the day the samples were taken, the TOC and DOC sample water was preserved. The TOC samples were

poured into 40 ml acid washed glass vials and preserved to a pH of 2 with 40 µl of 6 N HCl. The samples were then stored at 4ºC for a maximum of 2 weeks before analysis. The DOC samples were filtered through a glass fiber filter (Whatman GF/F filter with 0.7 µm retention). The filters were pre-washed with 20-30 ml of E-pure water. Then, the sample was passed through the filter. The first 5-10 ml of the sample was discarded and the next 30 ml filtered into a 40 ml acid washed glass vial. The samples were then preserved to a pH of 2 in the same manner as the TOC samples and stored at 4ºC for up to 2 weeks before analysis.

3.2.8.1 Standard Preparation

The TOC and DOC of the water samples were measured with a Shimadzu TOC-5000A (Shimadzu Corp., Kyoto, Japan). The TOC and DOC analyzer utilizes a three point calibration curve made with potassium hydrogen phthalate.

First, a stock primary standard of 1000 mg/L was prepared: 0.75 grams of Potassium Hydrogen Phthalate was dried in 103-110ºC oven for 30 minutes and cooled in a desiccator for an additional 30 minutes. Following the cooling process, 0.5314 grams of the dried Potassium Hydrogen Phthalate was weighed using an analytical balance, and added to a 250 ml volumetric flask filled half way with E-pure water. The 250 ml flask was then brought up to the mark with E-pure water. The stock primary solution of 1000 mg/L was put in a brown glass bottle and stored at 4ºC for a maximum of 3 weeks.

Second, an intermediate standard of 100 mg/L was made. To make the intermediate standard, 10 ml of the primary stock standard was transferred into a 100 ml volumetric flask half filled with E-pure water. The 100 ml flask was then bought up to the mark with additional E-pure water. The intermediate standard of 100 mg/L was stored at 4 degrees Celsius for a maximum of 2 days.

The working standards used in the calibration curve were 10, 5, 2, 1, and 0 mg/L. For each working standard, a 100 ml flask was filled half way with E-pure water and 100 µl of 6 N HCl was added to each flask. Then, the appropriate volume of intermediate standard was added to each flask as seen in Table 3. Lastly, the volume in each flask was brought up to mark with E-pure water.

Table 3: Working Standards for TOC/DOC Analysis

Working Standard (mg/L)	Volume of Intermediate Stock Added (ml)
10	10
5	5
2	2
1	1
0	0

3.2.8.2 TOC/DOC Quantification

Once all of the working standard were prepared, the auto-sampler cells for the Shimadzu TOC-5000A were filled. Each standard and sample was inverted three times, poured into an autosampler vial, and then the vial was covered with parafilm and plastic Shimadzu lids. The standards were placed in the the inner ring of the autosampler rack from highest to lowest, and then the samples were placed in the outer ring of the autosampler rack. Quality control was established by placing two working standards with known concentrations in with the sample vials to verify accurate measurements.

All standards and samples were sparged for five minutes before analysis to remove any carbon dioxide and then analyzed three to five times. The standards and samples were measured a minimum of three times, after which the standard deviation and coefficient of variation were calculated. If the values were not in the desired range (200 for standard deviation and 2.0% for coefficient of variation) after the third measurement, then another measurement was taken. Measurements were taken until the values were in the desired range or until 5 measurements were taken. Three calibration curves were produced and the instrument selected the best curve for determining the concentration of each sample.

3.3 Statistical Analysis

Two statistical methods were utilized for analyzing data collected from the sampling sites: correlation analyses and ANOVA analyses. Correlation analyses were

performed on the individual water quality parameters to identify relationships between them. ANOVA analyses were completed to determine differences between different sites and differences between season with each water quality parameter as well as a complied binary data set for several of the water quality parameters.

3.3.1 Correlation Analysis

Correlation analyses were done using the Microsoft Excel data analysis tool pack. The data analysis yields an R-value output which is a correlation coefficient representing the linear relationship between the data pairs. Correlation coefficient values range from -1.00 to +1.00, where the negative sign indicates an inverse correlation. Zero indicates no correlation and 1 indicates complete correlation.

The R-value is used to determine whether a correlation is statistically significant. The two factors that determine whether an R-value shows statistical significance is the confidence interval used and the number of data pairs that the R-value is generated from. The confidence interval used was a 95% confidence interval, which is commonly used for research. This is a P-value of 0.05. For example, a statistically siginificant correlation is a P-value of 0.05, and a highly significant correlation would be $\geq$0.005. A statistically significant R-value can be found on a correlation coefficient table with a P-value of 0.05 and the correct number of data points for each parameter.

3.3.2 ANOVA Analysis

The analysis of variance (ANOVA), also known as the F-test, is a method to determine the variation of the means of a group of data or variables to evaluate statistical significance. ANOVA analyses were done utilizing the Microsoft Excel data analysis tool pack. The ANOVA test assumes a null hypothesis, which states that there is no difference between the data within a data set. If the analysis is found to be statistically significant, then the null hypothesis is rejected for the alternative hypothesis. The alternative hypothesis states that the means of the data in the data set are different. Similar to the correlation analysis, a 95% confidence interval was considered to be statistically significant. A P-value of $\geq$0.05 would classify as statistically significant. The ANOVA analysis was performed with the data segregated by site to determine

differences in water quality between the different locations. Then the analysis was repeated with the data organized by season to assess seasonal differences in water quality.

3.3.3 Binary Data Sets

Binary data sets were constructed for further analysis of the data. The binary data sets were based upon 1 being defined as "polluted" and 0 being defined as "not polluted". For several of the water quality parameters, a benchmark pollution level was defined. Any measurement greater than the benchmark pollution level was represented with a 1, and any measurement less than the benchmark pollution level was represented with a zero. The binary data sets were then analyzed with the ANOVA analysis procedures discussed in the previous section.

4.0 Results

Water quality parameters, including indicator organisms, organics, and particles, were measured over four seasons at seven sampling sites in the West Boylston Brook tributary. This tributary feeds into the Wachusett Reservoir, which serves as a drinking water source for the Boston area. This chapter first presents information on the sampling sites. Then, variations in water quality at the different sampling locations and during different seasons are presented. Last, correlations among water quality parameters are assessed based on statistical relationships between parameters and their ability to assess pollution levels.

4.1 Sampling Site Descriptions

The seven sampling locations in the West Boylston Brook were chosen based on isolation of potential sources of microbial contamination to the brook. Table 4 lists the sites from the most upstream to the most downstream sampling location along with brief site descriptions. Figure 1 shows classified land uses within the West Boylston Brook watershed. All of the sampling sites are located between the headwater of the brook, Carroll's Pond, and the Wachusett Reservoir as seen in Figure 2.

Table 4: Sampling Site Descriptions

Site	Description
Site F	Downstream of agricultural land, dairy operation
Site E	Forested, downstream of pond, wildlife likely
Site D	Within a residential development
Site C	Branch downstream of a steep slope and to land characterized by commercial and residential development
Site B	Branch downstream of residential development
Site A	Downstream where branches join from Sites B and C
Gate 25	Approximately 100 yards before emptying into reservoir

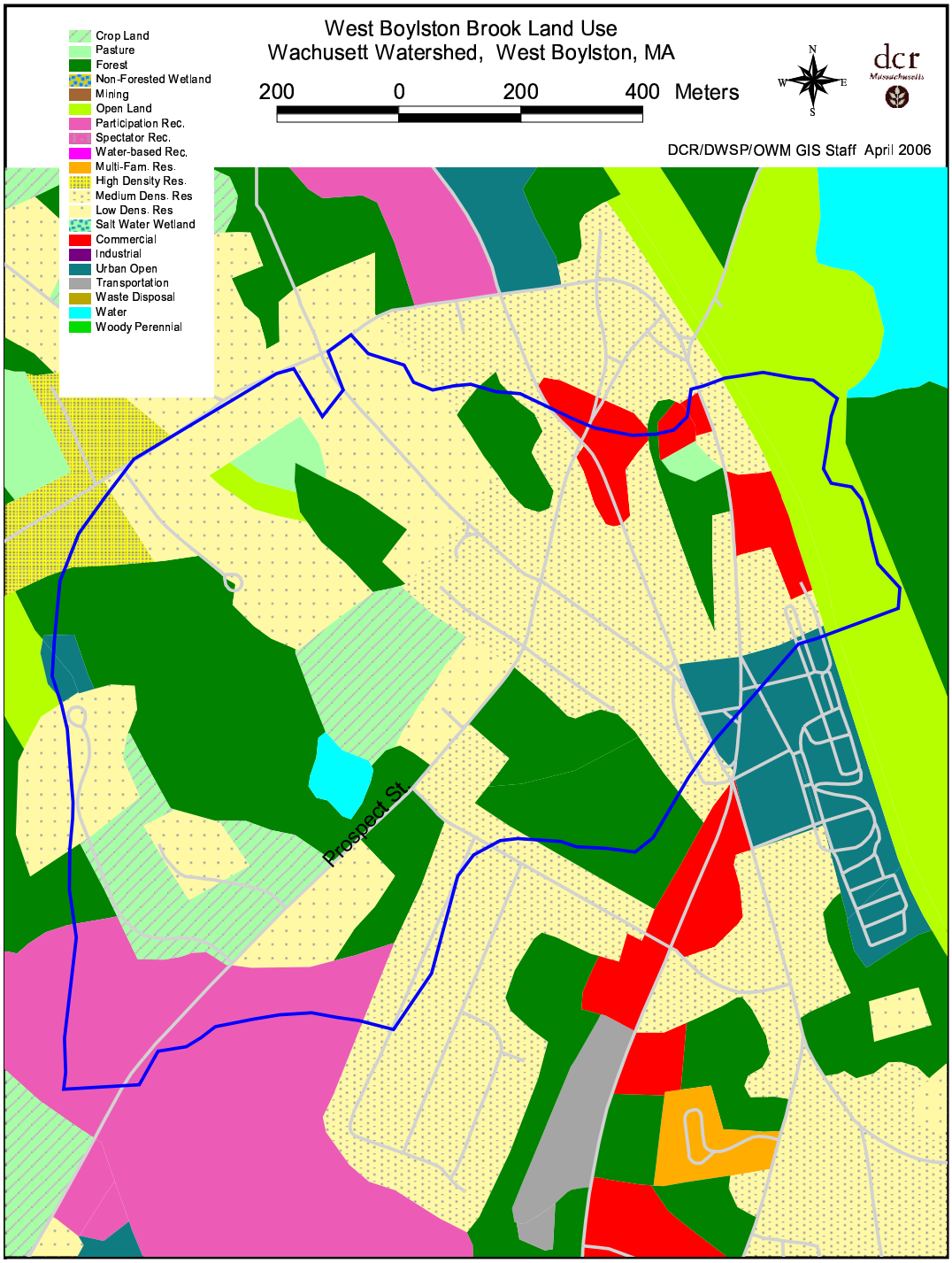

Figure 1: Land Uses in the West Boylston Brook Subbasin

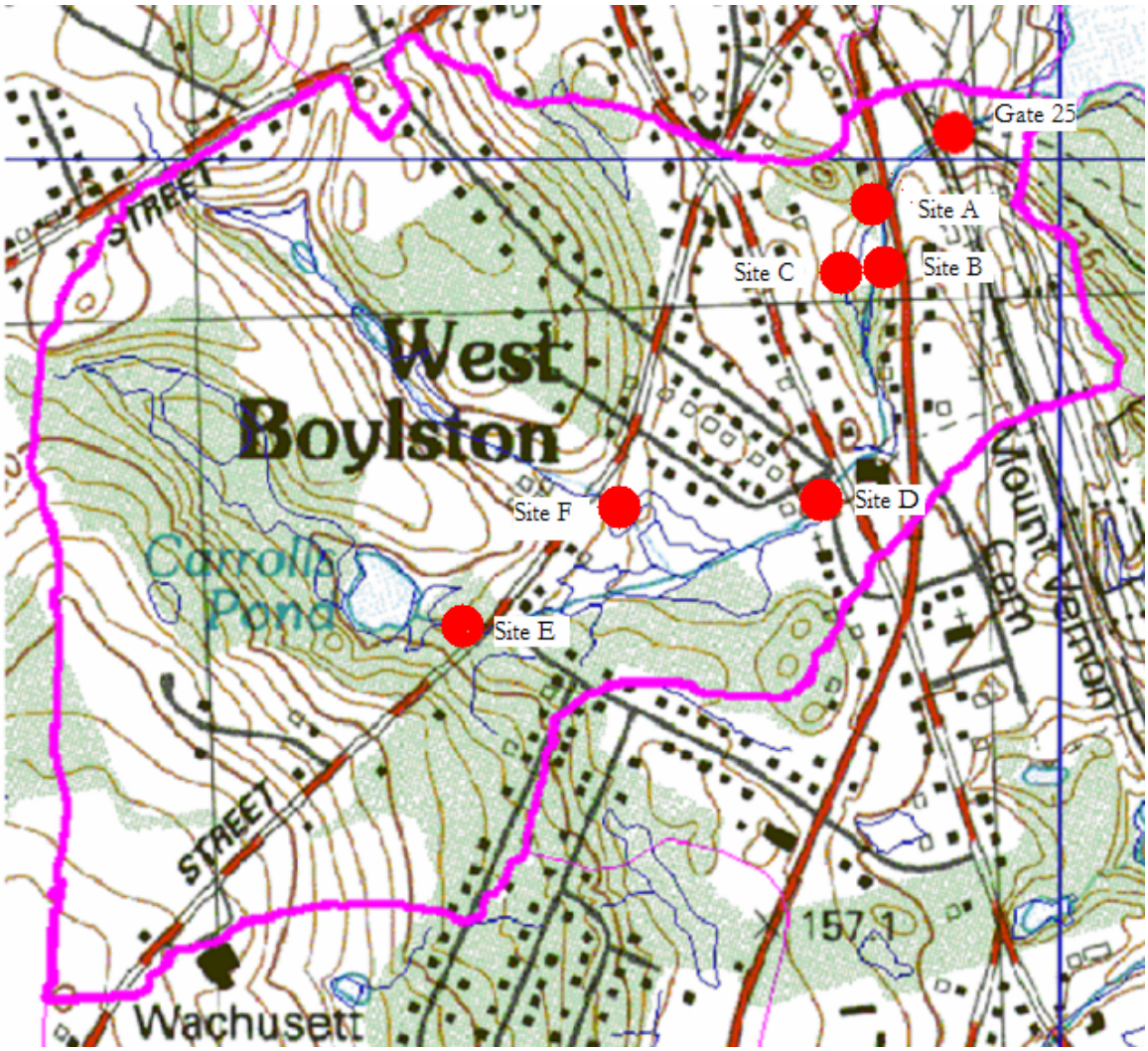

Figure 2: Sampling Locations in the West Boylston Brook Subbasin

Site F is not in the main channel of the West Boylston Brook. Site F is in a channel that enters the main channel between sites E and D and is located about 50 yards away from Site E. The site is downstream of agricultural land including a small dairy operation. This site is accessed from Prospect Street and is about 20 yards off the road. As shown in Figure 3, there is board crossing the brook. Samples were collected immediately upstream of the board. Flow was present at Site F throughout the year.

Figure 3: Photograph of Site F

Site E is the most upstream location in the main channel of the West Boylston Brook, located about 30 yards downstream of Carroll's Pond. The site is forested and it is likely that wildlife are present in the vicinity. The site was accessed from Prospect St. As seen in Figure 4, there are two large stones crossing the brook. Samples were collected immediately upstream of the stones. Flow was present at Site E throughout the year.

Figure 4: Photograph of Site E

Site D is located several hundred feet upstream of Sites A, B, and C in the main channel of the West Boylston Brook. It was accessed from Newton St. which is about 10 yards downstream of the sampling location. The site is in a residential development area.

The sampling location is at a large willow tree in the center of a yard as seen in Figure 5. Samples were collected directly in front of the willow tree. Flow was present at Site D throughout the year except in August 2005 when there was no flow at this location.

Figure 5: Photograph of Site D

Sites A, B, and C were accessed from West Boylston St. directly across from one of the Town of West Boylston's highway department buildings. Site C is located in a channel that feeds into the main West Boylston Brook channel downstream of Site B and upstream of Site A. The sampling location is about 50 yards upstream of Site A. Samples were collected just downstream of a steep slope at a large curvature in the channel as seen in Figure 6. The sampling location is in a wooded area downstream of commercial and residential development in the Central Street area. Flow was present throughout the year at Site C.

Figure 6: Photograph of Site C

Site B is located in the main channel of the West Boylston Brook about 60 yards upstream of Site A. The sampling location is in a wooded area downstream of residential development. The sampling location is near a large maple tree at a bend in the brook about 20 yards away from West Boylston Street. Samples were collected next to the maple tree upstream of the bend as seen in Figure 7.

Figure 7: Photograph of Site B

Site A is located where the channel that Site C is located in merges with the main channel of the West Boylston Brook. The sampling site is about 10 feet upstream of a conduit that carries the brook about 30 yards under West Boylston St. and the adjacent railroad tracks. Samples were collected at the bend in the stream just off the end of a

concrete bound laying in the stream bed as seen in Figure 8. Flow was present at Site A throughout the year.

Figure 8: Photograph of Site A

The Gate 25 sampling location is a location that had been used previously by the DCR for water quality sampling representative of the West Boylston Brook. The site is where the water exits the conduit that carries it under West Boylston St., and is approximately 100 yards upstream of where the brook enters the Wachusett Reservoir. There is a weir and a stage gage set up at the sampling location and is where stage readings were obtained for this study. Samples were collected about 1 meter downstream of the weir as seen in Figure 9. There was flow present at Gate 25 throughout the year.

Figure 9: Photograph of Sampling Location at Gate 25.

4.2 Sampling Dates

Ten sampling events were conducted between February 2005 and February 2006. As shown in Table 5, this included three winter, two spring, three summer and two fall events. These sampling events were chosen to help understand water quality variability resulting from seasonal differences. Also shown in Table 5 are precipitation conditions for each sampling event. Dry and wet weather flows were sampled to aid in analyzing contamination levels in the brook. The complete set of water quality parameters was analyzed for sampling dates from June 2005 to February 2006. Prior to June of 2005, organic carbon measurements and particle counts were not taken.

Table 5: Sampling Date with the Season the Sampling Represents

Sampling Date	Season
February 23, 2005	Winter
March 30, 2005	Spring
May 24, 2005 *	Spring
June 22, 2005	Summer
July 20, 2005	Summer
August 22, 2005	Summer
October 23, 2005 *	Fall
November 29, 2005	Fall
January 24, 2006	Winter
February 18, 2006	Winter

*** indicates sampling was conducted during a rain event**

4.3 Variations in Water Quality Parameters among Sampling Locations

Several water quality parameters showed variations at the different sampling locations on a given sampling date. One of the sampling events that shows many of the prevalent trends clearly is October 23, 2005. The following sections present water quality at the seven sampling sites for this sampling date. Data from other sampling dates are also summarized to demonstrate trends in water quality with location in the West Boylston Brook tributary.

4.3.1 Chemical Water Quality Parameters by Site

Dissolved oxygen concentrations at the seven sampling sites are shown in Figure 10, along with the temperature. The dissolved oxygen concentration tended to increase towards the more downstream locations. Dissolved oxygen levels on October 23, 2005 ranged from a low of 9.9 mg/L at Site E to a high of 11.2 mg/L at Gate 25. The dissolved oxygen at Gate 25 was 13% higher than at Site E. The temperature ranged from 9.0°C at Sites D and E to 9.8°C at Site C.

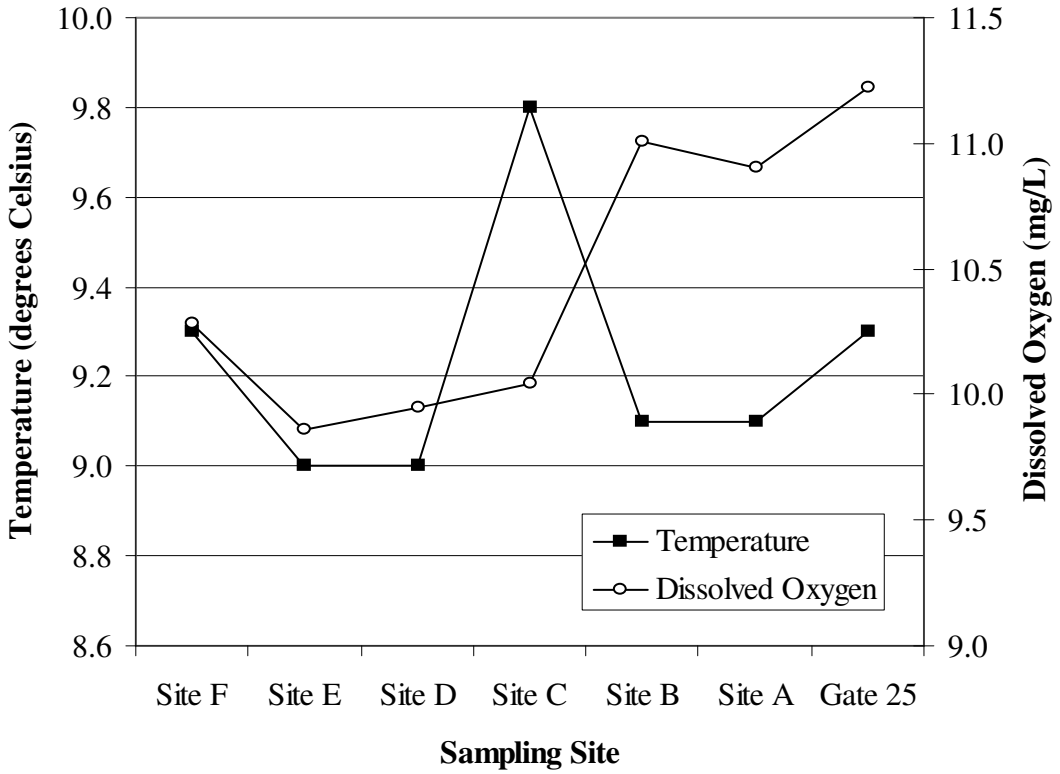

Figure 10: Temperatures and Dissolved Oxygen Levels on October, 23 2005

The high temperature at Site C is most likely due to groundwater influences on the brook. Seasonally, the temperature at Site C is higher in the winter compared to the other six sites, and lower in the summer than at any of the other sampling locations.

The characteristics of the stream-flow in the brook have a direct affect on the re-aeration rate of the water which in turn affects the amount of dissolved oxygen that will be present. Table 6 lists the ranges of dissolved oxygen observed over the course of the entire sampling period along with a listing of the sites with the highest and lowest dissolved oxygen levels. Sites C and E tended to have low dissolved oxygen levels in comparison to other sampling locations. Gate 25, Site B, and Site F tended to have high dissolved oxygen levels in comparison to the other sampling locations.

Table 6: Dissolved Oxygen Ranges

Sampling Date	DO Range (mg/L)	Site of Lowest DO	Site of Highest DO
Feb. 05 (Winter)	10.6 - 12.9	Site C	Gate 25
Mar. 05 (Spring)	11.2 - 12.6	Site C	Gate 25
May 05 (Spring)	10.5 - 11.8	Site E	Site B
Jun. 05 (Summer)	8.3 - 10.3	Site C	Site F
Jul. 05 (Summer)	5.9 - 8.0	Site E	Site B
Aug. 05 (Summer)	7.3 - 9.6	Site C	Site F
Oct. 05 (Fall)	9.9 - 11.2	Site E	Gate 25
Nov. 05 (Fall)	7.4 - 10.1	Site C	Gate 25
Jan. 06 (Winter)	9.7 - 13.4	Site C	Gate 25
Feb. 06 (Winter)	10.3 - 14.0	Site C	Gate 25

The flow characteristics at Gate 25, Site B, and Site F may have contributed to the elevated levels of dissolved oxygen measured at these locations. The dissolved oxygen content of the water at Gate 25 is measured just downstream of a sharp crested, v-notch weir which causes significant mixing and re-aeration. The flow at sites B and F is fast moving compared to the other sites due to a steeper slope at these locations. Also, the channel bottom at sites B and F is rocky, which contributes to mixing and thus reaeration at these sites.

Site E tended to have the lowest levels of dissolved oxygen which is probably due to the nature of the flow at that site. The water is very slow moving with a smooth silt covered channel bottom due to some ponding in the stream. There appears to be little reaeration of the water at Site E. Site C may have low levels of dissolved oxygen due to a low flow at this location or due to groundwater influences on the brook.

Conductivity and pH levels at the seven sampling sites on October 23, 2005 are shown in Figure 11. The pH and conductivity appear to follow similar trends for most sites. The conductivity in the brook ranged from 110 µS at Site C to 203 µS at Site F, a 46% difference. There was a modest increase in conductivity from Site C downstream to

Site Gate 25; however no apparent trend at the upstream sites. The pH ranged from 6.71 at Site C to 7.39 at Gate 25.

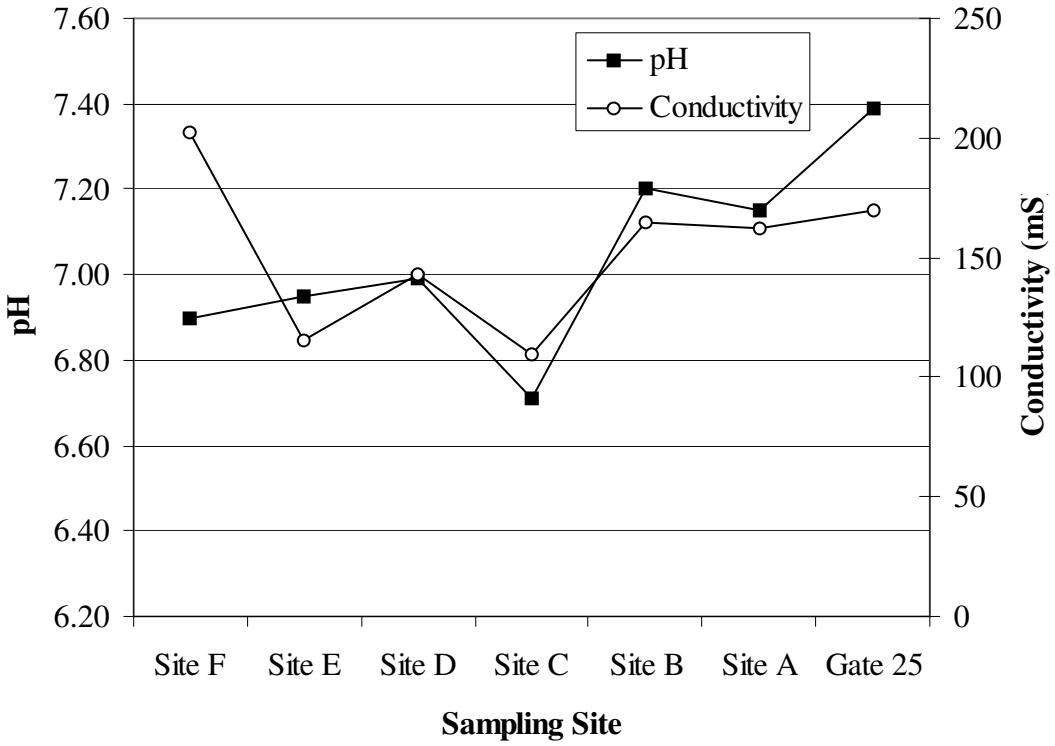

Figure 11: pH and Conductivity on October 23, 2005

Table 7 lists the range of conductivity measured during each sampling event, along with the sites which had the highest and lowest values. Site E tended to have low conductivity levels in comparison to the other sample locations. High conductivity levels were sporadically found at several of the other sampling locations. The high conductivities were expected in downstream sites around more developed areas. Inorganic suspended solids from runoff as well as the presence of chloride and nitrate from failing septic systems will increase conductivity levels.

Table 7: Conductivity Ranges

Sampling Date	Conductivity Range (µS)	Site of Lowest Conductivity	Site of Highest Conductivity
Feb. 05 (Winter)	536 - 189	Gate 25	Site D
Mar. 05 (Spring)	123 - 430	Site E	Site C
May 05 (Spring)	194 – 356	Site E	Site B
Jun. 05 (Summer)	206 - 855	Site E	Gate 25
Jul. 05 (Summer)	205 - 630	Site E	Gate 25
Aug. 05 (Summer)	144 - 790	Site E	Gate 25
Oct. 05 (Fall)	110 - 203	Site C	Site F
Nov. 05 (Fall)	107 - 426	Site E	Gate 25
Jan. 06 (Winter)	95 - 369	Site E	Site C
Feb. 06 (Winter)	96 - 492	Site E	Site C

4.3.2 Particulate Matter by Site

Turbidity levels and particle counts at each site tended to follow similar trends. Sites with high turbidity readings also had high particle counts and visa versa, as seen in Figure 12 for the October 23, 2005 sampling event. On this date, site C had the highest turbidity level of 3.18 ntu and the highest particle count of 6583 particles/ml. The site that had the lowest turbidity was Site E with 1.16 ntu while the site with the lowest particle count was Site F with 3228 particles/ml.

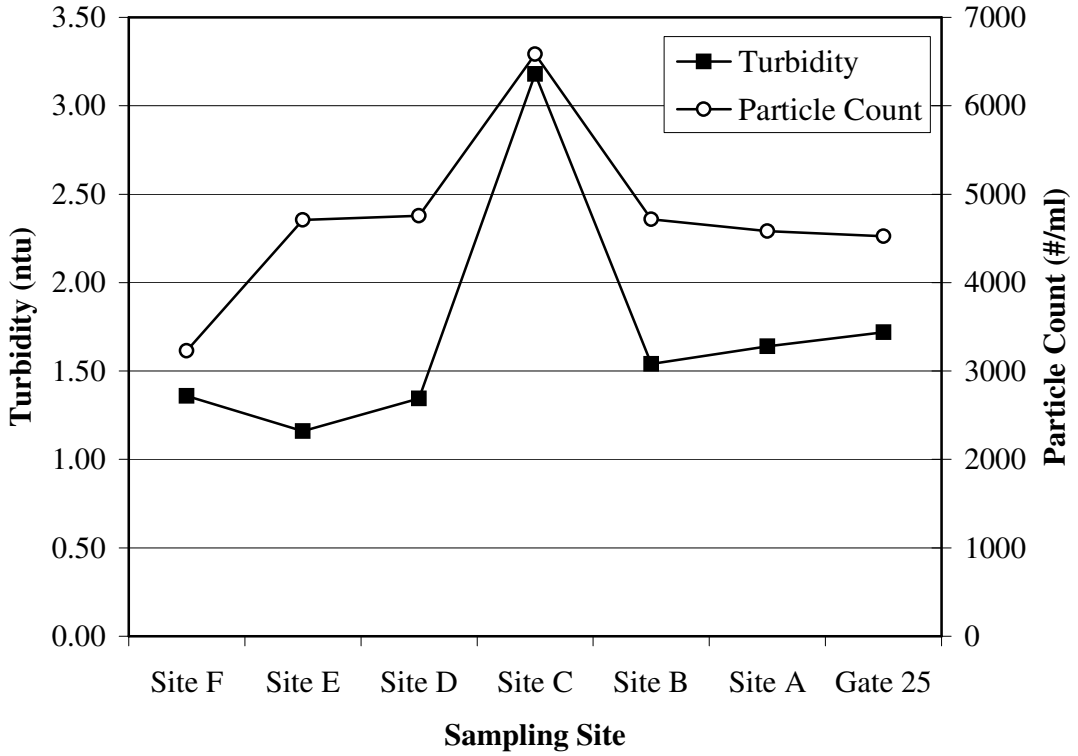

Figure 12: Turbidity and Particle Counts on October 23, 2005

Table 8 lists the turbidity ranges and sites with the highest and lowest turbidity values. Sites B and E tended to have lower turbidity in comparison to the other sampling locations, and sites A, C, and D tended to have higher turbidity in comparison to the other sampling locations. However, high turbidity readings at Site C may be due to sample collection problems. Often times Site C had comparably low flow which made collecting a sample difficult. Any disturbance to the channel bottom when sampling can increase a turbidity reading; this may have been a factor at Site C.

Sites E and B tended to have low turbidity levels which may be due to the nature of flow at these sites. Sites E and B both had flow that was not turbulent and did not cause a lot of mixing. Due to the nature of the flow at these sites the suspended solids may settle at a greater rate thus yielding lower turbidity readings.

Table 8: Turbidity Ranges

Sampling Date	Turbidity Range (ntu)	Site of Lowest Turbidity	Site of Highest Turbidity
Feb. 05 (Winter)	0.80 - 2.37	Site E	Site D
Mar. 05 (Spring)	1.66 - 17.20	Site E	Site C
May 05 (Spring)	1.92 - 11.50	Site B	Site C
Jun. 05 (Summer)	0.21 - 8.37	Site B	Site D
Jul. 05 (Summer)	0.27 - 3.01	Site B	Site E
Aug. 05 (Summer)	0.35 - 3.46	Gate 25	Site F
Oct. 05 (Fall)	1.16 - 3.18	Site E	Site C
Nov. 05 (Fall)	0.64 - 2.47	Site B	Site A
Jan. 06 (Winter)	0.56 - 2.97	Site F	Site A
Feb. 06 (Winter)	0.89 - 1.94	Site B	Site A

4.3.3 Organic Matter by Site

Data was collected on three measurements of organic matter: total organic carbon (TOC), dissolved organic carbon (DOC), and ultraviolet light absorbance at 254 nm (UV_{254}). The data from October 23, 2005 are shown in Figure 13. As demonstrated in this figure, the three organic matter measurements showed similar patterns with regard to site. The highest organic carbon levels were found at Site C with a TOC of 7.91 mg/l, a DOC of 7.53 mg/l, and a UV_{254} absorbance of 0.229 cm^{-1}. The lowest organic carbon levels were found at site E with a TOC of 5.27, a DOC of 4.75 mg/l, and UV_{254} of 0.164 cm^{-1}. Organic matter concentrations overall were lower at the upstream sites (F and E), slightly elevated at Site C, and consistent from Site B to Gate 25.

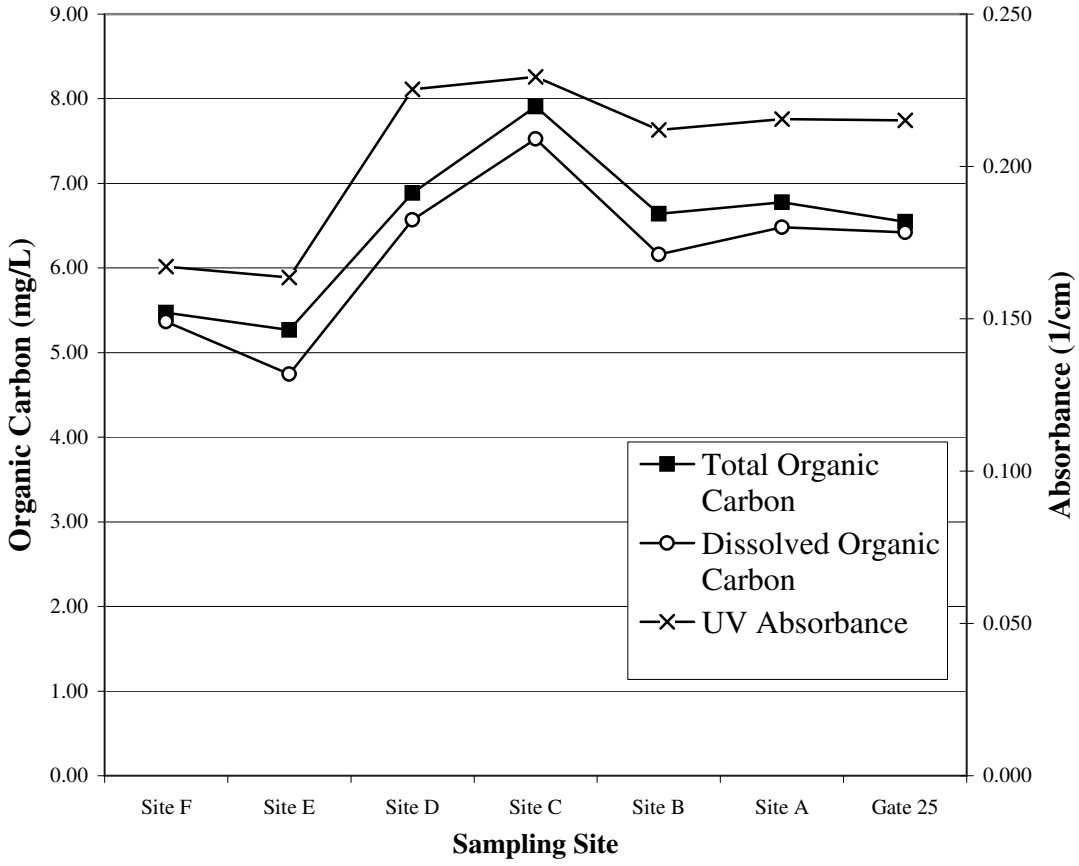

Figure 13: Organic Carbon and UV_{254} Absorbance Levels on October 23, 2005

Table 9 lists the range of TOC observed on each of the sampling dates and where levels where highest and lowest. Unlike October 2005, Site C tended to have the lowest levels of TOC during the other summer, fall, and winter sampling events. Site E had the highest levels of TOC on four of the seven sampling events. This was not unexpected as Site E is located just downstream of a wooded wetland.

Table 9: Total Organic Carbon Ranges

Sampling Date	Total Organic Carbon Range (mg/L)	Site of Lowest Total Organic Carbon	Site of Highest Total Organic Carbon
Feb. 05 (Winter)	Not Measured		
Mar. 05 (Spring)	Not Measured		
May 05 (Spring)	Not Measured		
Jun. 05 (Summer)	1.04 - 3.97	Site C	Site E
Jul. 05 (Summer)	1.26 - 5.49	Site C	Site E
Aug. 05 (Summer)	1.32 - 4.06	Gate 25	Site E
Oct. 05 (Fall)	5.27 - 7.91	Site E	Site C
Nov. 05 (Fall)	5.09 - 7.92	Site C	Site D
Jan. 06 (Winter)	2.77 - 3.42	Site C	Site D
Feb. 06 (Winter)	2.04 - 3.07	Site C	Site E

4.3.4 Indicator Organisms by Site

Two biological indicator organisms, fecal coliforms and *E. coli,* were measured to assess possible locations where microbial contamination was entering the West Boylston Brook. Concentrations of the indicators for the October 23, 2005 sampling date are shown in Figure 14. In general, the two indicators tended to vary by site in a similar fashion. This is to be expected as *E. coli* are a subset of the fecal coliform bacteria group. On October 23, 2005, the fecal coliforms ranged from 42 cfu/100 ml at Site E to 1322 cfu/100 ml at Gate 25. *E. coli* levels ranged from 42 cfu/100 ml at Site E to 861 cfu/100 ml at Gate 25.

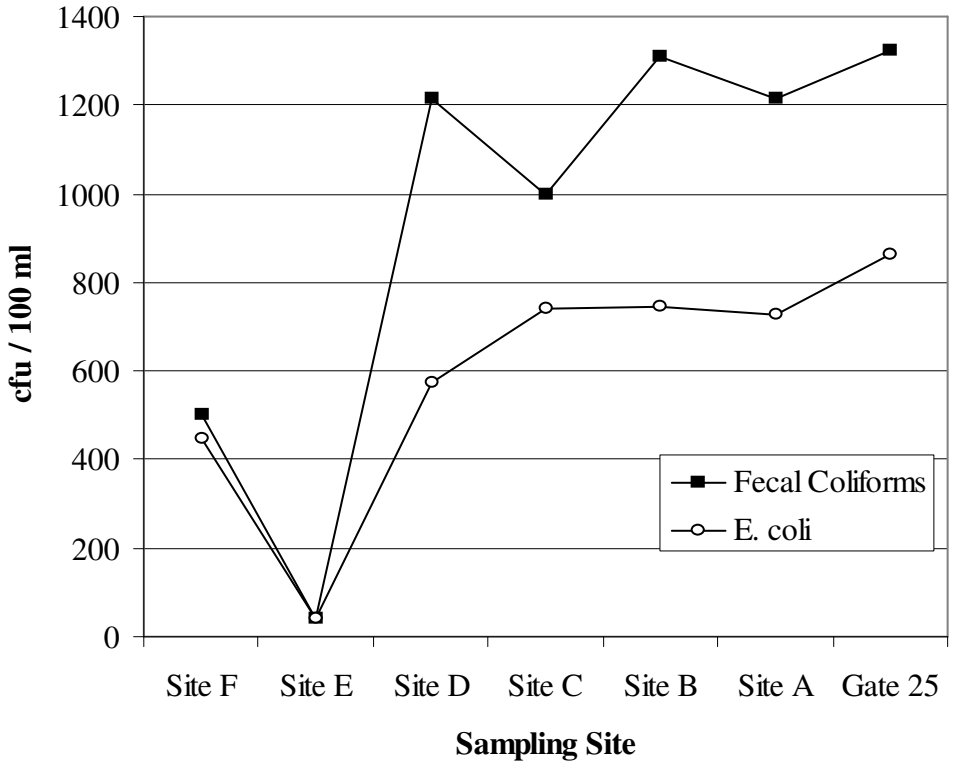

Figure 14: Fecal Coliform and *E. coli* Concentrations on October 23, 2005

Table 10 lists the fecal coliform ranges observed on each sampling date and the sites which had the extreme values. Site E tended to have the lowest number of fecal coliforms. Site E was expected to have low levels of fecal coliforms because it is just downstream of wooded, undeveloped land where human influence is minimal. The fecal coliforms at this location are most likely due to wildlife sources. Site C had the highest number of fecal coliforms on five of the ten sampling dates. This site is a small tributary channel to the main West Boylston Brook channel, and demonstrates that fecal contamination may arise from that area of the subbasin watershed. High levels were also sporadically observed at Site F, Site D, and Gate 25. Site F is influenced by a dairy operation and Site D by a residential development.

Table 10: Fecal Coliform Ranges

Sampling Date	Fecal Coliform Range (cfu/100ml)	Site with Lowest Number of Fecal Coliforms	Site with Highest Number of Fecal Coliforms
Feb. 05 (Winter)	7 - 64	Site C	Site D
Mar. 05 (Spring)	14 - 410	Site E	Site C
May 05 (Spring)	450 - 4800	Site E	Site C
Jun. 05 (Summer)	35 - 387	Site E	Gate 25
Jul. 05 (Summer)	85 - 377	Site E	Site F
Aug. 05 (Summer)	280 - 1109	Gate 25	Site C
Oct. 05 (Fall)	42 - 1322	Site E	Gate 25
Nov. 05 (Fall)	97 - 2667	Site E	Site F
Jan. 06 (Winter)	141 - 487	Sites E and F	Site C
Feb. 06 (Winter)	8 - 787	Site E	Site C

4.4 Variation in Water Quality Parameters by Season

The water quality parameters discussed in Section 4.3 were also analyzed by season to determine impacts of seasonal changes on pollutant levels. Some of the water quality parameters show strong seasonal trends while other water quality parameters do not vary significantly during the year. The water quality measurements from Gate 25, which are presented in the following sections, show a representation of the entire sub-watershed basin and how the seasonal changes affect the water quality of the basin.

4.4.1 Chemical Water Quality Parameters by Season

Temperature and dissolved oxygen followed clear seasonal trends, as seen in Figure 15. Temperature was greater in the summer months than the winter months. The temperature at Gate 25 ranged from 2.2°C in February of 2006 to 15.0°C in July and August of 2005. The other sites followed the same seasonal patterns.

The dissolved oxygen concentration was inversely related to the temperature. This was expected as saturation values of dissolved oxygen decrease with increasing

temperature. Dissolved oxygen at Gate 25 ranged from 8.1 mg/l in July of 2005 to 14.0 mg/L in February of 2006. There were similar seasonal temperature and dissolved oxygen trends at the other six sampling locations.

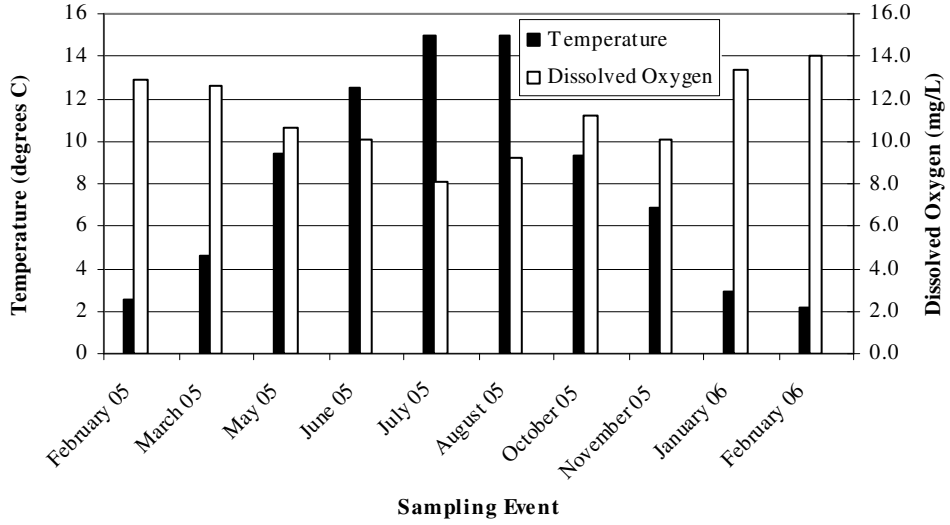

Figure 15: Temperature and Dissolved Oxygen Concentration at Gate 25

The conductivity and pH measured at Gate 25 over the four seasons is shown in Figure 16. The pH was reasonably consistent throughout the sampling period. The lowest pH of 6.8 occurred in May of 2005 and the highest pH of 7.5 occurred in February of 2006. There were no clear seasonal trends at any of the seven sampling locations.

Also shown in Figure 16 is the conductivity at Gate 25. Conductivity ranged from 170 µS in October of 2005 to 855 µS in June of 2005. At Gate 25, the conductivity was higher in the summer months than the rest of the year. The highest summer conductivity was 855 µS in June of 2005, while the highest winter conductivity was 536 µS in February of 2005. In the spring, conductivity was highest in March 2005 at 372 µS, and in the fall was 426 µS in November 2005. All of the other sampling locations had similarly high conductivities in the summer months except Site C. Site C did not seem to show any seasonal trends. The conductivity stayed relatively constant throughout the year with an average of 417 µS, but peaked in February of 2005 to a level of 803 µS.

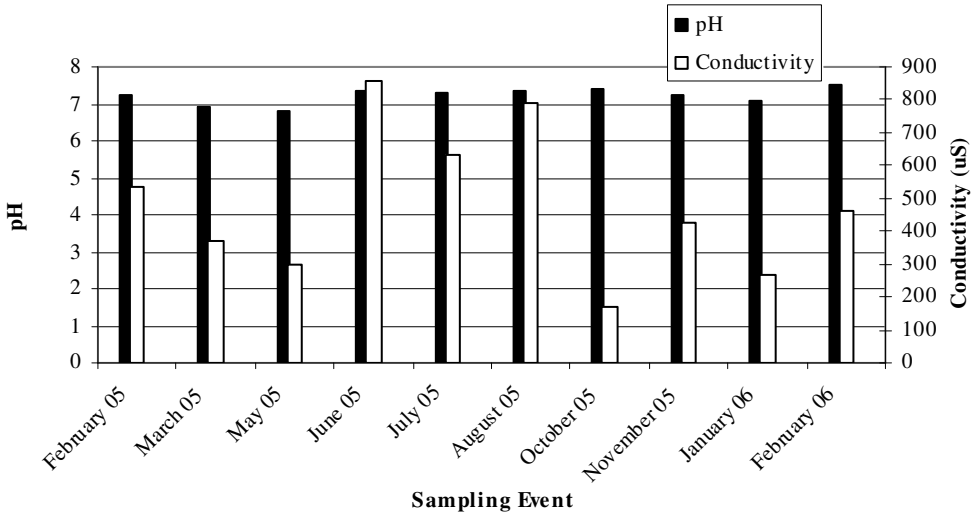

Figure 16: Conductivity and pH at Gate 25

4.4.2 Particulate Matter by Season

The turbidity and particle count data from Gate 25 are shown in Figure 17. Particle count data was available from June 2005 onward. Seasonal trends are not readily apparent at Gate 25 nor any of the other sampling locations. Over the 13-month sampling period, the turbidity ranged from 0.35 ntu in August of 2005 to 3.38 ntu in March of 2005. The lowest particle count at Gate 25 was 1032 particles/ml in August of 2005 and the highest particle count was 4524 particles/ml in October of 2005.

Although the turbidity and particle count data did not show seasonal trends, these two parameters where thought to be dependent upon climate or more specifically precipitation conditions and flow conditions. Sampling in May 2005 and October 2005 was conducted during rainfall events. There was higher turbidity at Gate 25 in May 2005 and October 2005 in comparison to other sampling events, but the trend was not as apparent as expected. Other sites were similar with regard to particulate matter, in that a trend between rainfall data and the two particle measurements was not apparent.

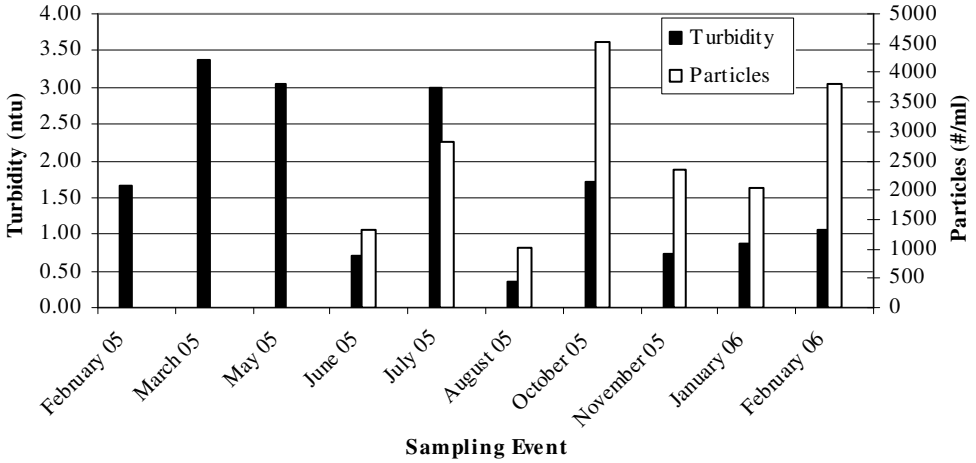

Figure 17: Turbidity and Particle Counts at Gate 25

4.4.3 Organic Matter by Season

Organic carbon data were measured from June of 2005 through February of 2006. As shown in Figure 18, organic carbon levels were higher in the fall than in the winter and summer months. The highest TOC concentration was observed in November of 2005 at 6.62 mg/l. In the summer, TOC values ranged from 1.28 mg/l to 2.87 mg/l, while in the winter levels ranged from 2.31 mg/l to 3.34 mg/l. The TOC on January 24, 2006 is about 50 percent lower than the total organic carbon on November 29, 2005.

The DOC and UV_{254} levels at Gate 25 followed the same seasonal trends as the TOC. The highest DOC concentration was 6.42 mg/l in October of 2005 and the lowest DOC concentration was 1.28 mg/L in June of 2005. The highest UV_{254} absorbance was 0.215 cm^{-1} in October of 2005, and the lowest UV_{254} absorbance 0.043 cm^{-1} in June of 2005. High organic carbon measurements in the fall months are most likely due to falling leaves entering the water, thus adding organic matter to the water.

All of the sampling locations had similar seasonal organic carbon trends except for Site E. At Site E, the TOC and DOC followed the same seasonal trends as the other sampling locations, but the UV_{254} did not. UV_{254} levels did not reveal any seasonal trend and were relatively high in the spring months when the TOC and DOC were

comparatively low. The UV_{254} at Site E ranged from 0.069 cm^{-1} in February of 2006 to 0.183 cm^{-1} in July of 2005.

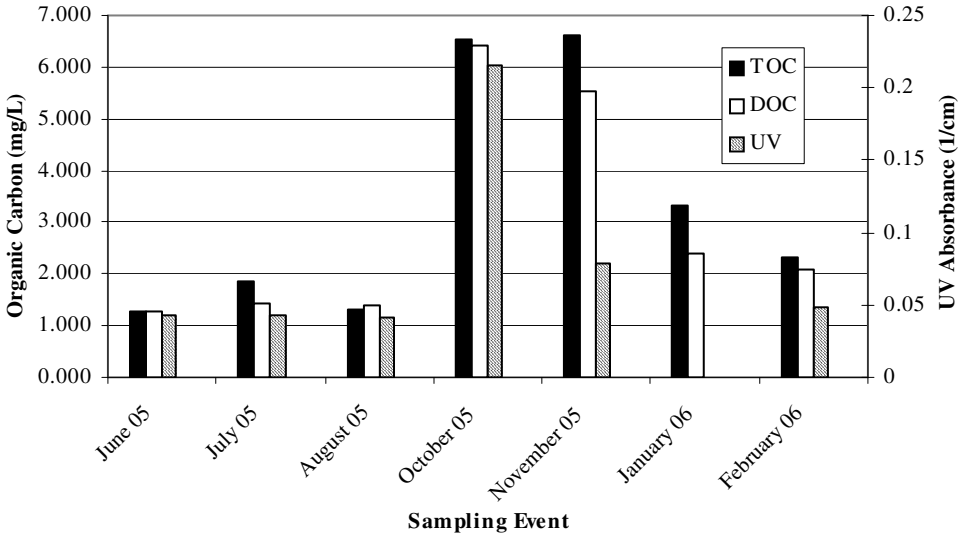

Figure 18: Organic Carbon Levels and UV_{254} Absorbance at Gate 25

4.4.4 Indicator Organisms by Season

Figure 17 presents the fecal coliform and *E. coli* concentrations at Gate 25 for the ten sampling events. The fecal coliforms range from 35 cfu/100 ml in March of 2005 to 1322 cfu/100 ml in October of 2005. The only apparent seasonal trend is that the fecal coliform levels are lower in the winter months. Lower levels were expected during the winter because cold temperatures cause microorganisms to die off at a faster rate. *E. coli* concentrations at Gate 25 range from 35 cfu/100 ml in March of 2005 to 861 cfu/100 ml in October of 2005.

Each of the other sampling locations had similar seasonal trends as Gate 25 excluding Site C. Fecal coliform and *E. coli* levels were much lower in the winter months, and there were spikes in indicator organism levels during various months outside of the winter season. All of the sampling sites except sites E and F had high levels in May and October which is most likely due to rain events during both of these sampling events.

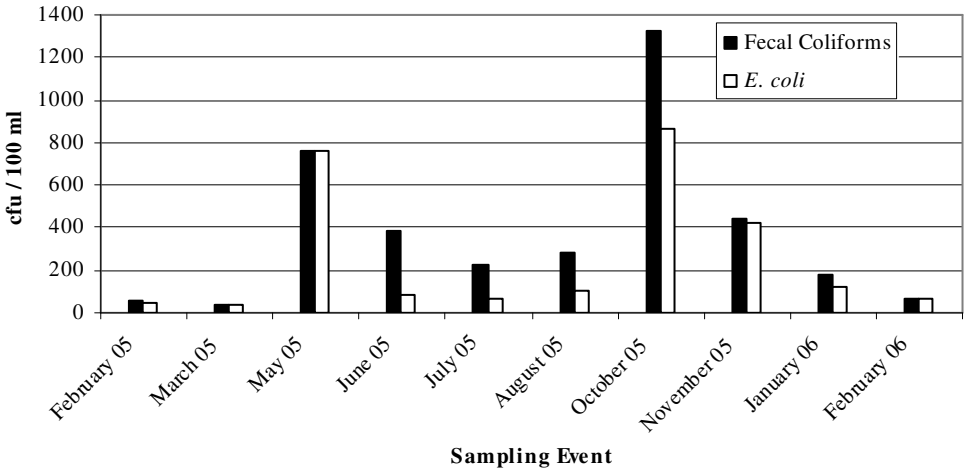

Figure 19: Fecal Coliform and *E. coli* levels at Gate 25

Site C yielded some interesting indicator organism data. The fecal coliform and *E. coli* concentrations at Site C were not lower in the winter months than the other seasons, as seen in Figure 20. The fecal coliforms at Site C ranged from 7 to 787 cfu/100 ml in the winter, from 410 to 4800 cfu/100 ml in the spring, from 191 to 1109 cfu/100 ml in the summer, and from 121 to 1000 cfu/100 ml in the fall. There was not a dramatic drop in indicator organisms at Site C during the winter months.

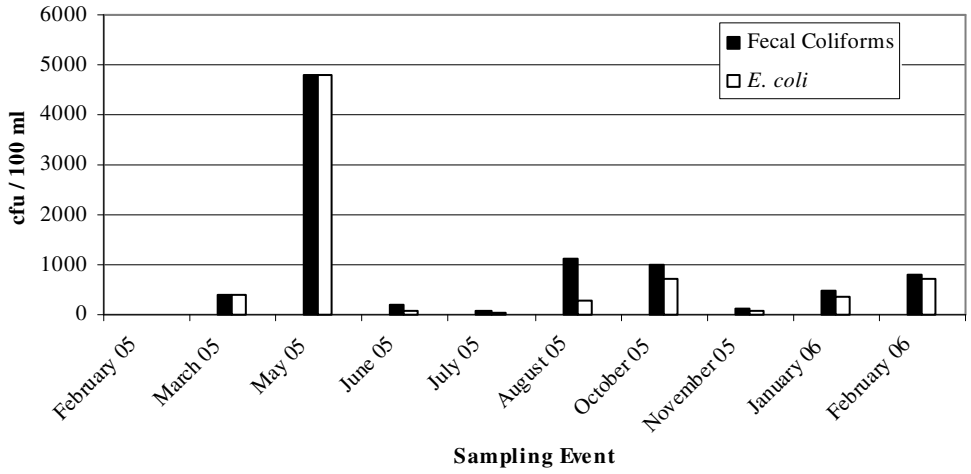

Figure 20: Fecal Coliform and *E. coli* Levels at Site C

4.5 Statistical Analysis

Several statistical analyses were performed on the water quality data collected throughout this study. The following sections present the data analyses, which were conducted using Microsoft Excel data analysis tools. First, a correlation analysis was performed to identify relationships between the water quality parameters. Second, ANOVA analysis was completed to determine differences between water quality at different sites and differences between seasons. Lastly, the water quality data were converted to a binary data set and analyzed with an ANOVA analysis to determine the relative capacity of water quality parameters to characterize the tributary as polluted or unpolluted.

4.5.1 Correlation Analysis

A correlation analysis was used to determine relationships between water quality parameters. The correlation analysis performed by Microsoft Excel provides an R-value as an output for each pair of water quality parameters. An R-value shows how well two water quality parameters are correlated with one-another. An R-value of 1 shows complete correlation, and an R-value of 0 shows no correlation. A negative R-value

indicates an inverse correlation, and similarly an R-value of -1 shows complete inverse correlation.

The correlation analysis was based on a 95% confidence level, or a P-value of 0.05. In order to determine if a statistically significant correlation exists, a minimum R-value must be determined from a correlation coefficient table. Minimum R-values are based upon the number of data points, n, which are evaluated. The number of data points in this study ranged from 41 data points (UV_{254}) to 65 data points (majority of the other parameters) and are summarized in Table 11. As shown in this table, for example, an R-value of 0.312 shows a statistically significant correlation between any of the other water quality parameters and UV_{254}.

Table 12 provides the Excel output for the correlation analysis of the water quality data collected in this study. Comparison of the R-values in this table to minimum values in Table 11 reveals which parameters have statistically significant correlations. The correlations are highlighted n bold in Table 12.

Table 11: Number Data Points for each Water Quality Parameter and the Minimum R-Values Needed Statistically Significant Correlation (95% Confidence Level)

Water Quality Parameter	Number of Data Points (n)	Minimum R-Value
Temperature	65	0.279
Dissolved Oxygen	65	0.279
Conductivity	65	0.279
pH	65	0.279
UV_{254}	41	0.312
TOC	48	0.294
DOC	48	0.294
Turbidity	65	0.279
Particles	48	0.294
FC	65	0.279
E. coli	65	0.279

Table 12: R-Values for Correlation Analysis on Water Quality Data

	Temp.	*DO*	*Cond.*	*pH*	*UV*	*TOC*	*DOC*	*Turbid.*	*Partic.*	*FC*	*E. coli*
Temp	1										
DO	**-0.763**	1									
Cond.	**0.282**	-0.163	1								
pH	-0.157	0.242	-0.097	1							
UV	0.077	0.107	**-0.607**	-0.005	1						
TOC	-0.254	0.095	**-0.619**	0.004	**0.728**	1					
DOC	-0.087	0.004	**-0.575**	-0.071	**0.835**	**0.965**	1				
Turbidity	0.093	0.057	0.004	-0.123	0.187	0.048	0.075	1			
Particles	-0.195	0.307	**-0.492**	0.265	**0.654**	**0.516**	**0.521**	**0.532**	1		
FC	0.071	-0.065	-0.160	-0.230	**0.357**	**0.468**	**0.469**	**0.360**	0.145	1	
E. coli	-0.020	-0.008	-0.160	-0.196	0.263	**0.525**	**0.470**	**0.397**	0.168	**0.965**	1

Overall there were 21 pairs of water quality parameters that were statistically correlated to one another (16 directly correlated and 5 inversely correlated) out of a possible 55 pairings. Table 13 lists all of the correlated pairs. pH was the only water quality parameter that did not show any correlation to any of the other water quality parameters.

Conductivity and dissolved oxygen were both correlated with temperature. Dissolved oxygen showed an expected inverse correlation to temperature. As temperature increases the saturation level of dissolved oxygen in water decreases which yields an inverse correlation between the two parameters.

The correlation between temperature and conductivity may be due to biological processes. Biological processes that affect conductivity (i.e. the degradation of organic matter) are affected by temperature and therefore may be the source of the correlation between temperature and conductivity. Conductivity was also inversely related to organic matter which was to be expected because organic matter does not carry a charge. Conductivity was also inversely correlated with particle counts. This inverse correlation shows that the majority of the particulate matter in the water was organic which supports the correlation between conductivity and temperature. Particle counts were also correlated to all of the organic carbon measurements, again showing that the particulate matter in the water was for the most part organic matter.

Turbidity was not correlated to any organic matter measurements even though turbidity was correlated with particle counts. The turbidity is not a measure of the

organic matter, but instead a measure of small inorganic dissolved particles. It is important to note that although turbidity and particle counts correlate to one another, they are representative of different types of particles.

Each of the organic carbon measurements and particulate matter measurements were correlated to each indicator microorganism with the exception that UV_{254} did not correlate with *E. coli*. These correlations are most likely because microorganisms can attach to solids found in water which increases their survival rates.

Table 13: Correlations between Water Quality Parameters

Direct Correlation	Inverse Correlation
Temperature + Conductivity	Temperature + Dissolved Oxygen
UV_{254} + TOC	Conductivity + UV_{254}
UV_{254} + DOC	Conductivity + TOC
UV_{254} + Particle Counts	Conductivity + DOC
UV_{254} + Fecal Coliforms	Conductivity + Particle Counts
TOC + DOC	
TOC + Particle Counts	
TOC + Fecal Coliforms	
TOC + *E. coli*	
DOC + Particle Counts	
DOC + Fecal Coliforms	
DOC + *E. coli*	
Turbidity + Particle Counts	
Turbidity + Fecal Coliforms	
Turbidity + *E. coli*	
Fecal Coliforms + *E. coli*	

4.5.1.1 Correlation Analysis with Stage Height

Further correlation analysis was carried out in the same manner as in the previous section to determine if any of the water quality parameters were correlated with stage height. There were 10 data points of stage height (1 for each sampling event), but 7 measurements at the 7 sites. An R-value of 0.632 or greater indicated a correlation. Stage height was found to be directly correlated with particles and organic carbon (TOC, DOC, and UV_{254}), and inversely correlated with conductivity. The R-values for each of the water quality parameters that had a correlation with stage height are listed in Table 14.

Table 14: R-Values for each Water Quality Parameter Correlated with Stage Height

Water Quality Parameter	R-value
UV_{254}	0.965
DOC	0.771
Particles	0.766
Conductivity	-0.762
TOC	0.711

4.5.2 ANOVA Analysis

ANOVA analysis was performed to determine the differences between sites and the differences between the seasons for each of the water quality parameters. For site analysis, the null hypothesis was that the mean of the data for each constituent in the sub-basin was the same at every site. For the seasonal analysis, the null hypothesis was that the mean of the data for each constituent was the same for every season. The ANOVA analysis was based upon a 95% confidence level or a P-value of 0.05. Thus, for a P-value of less than or equal to 0.05, the null hypothesis was rejected.

4.5.2.1 ANOVA Site Analysis

Differences in the eleven water quality parameters were analyzed according to site variations between the seven sampling sites. There were a different number of data

points at each site and for each water quality parameter. The specific numbers of data points are shown in Table 15. TOC, DOC, UV_{254} and particle counts have fewer data points because they were not measured until the June 2005 sampling event. Site D also has fewer data points because low or no flow prevented sampling on August 2005.

Table 15: Number of Data Points for the ANOVA Site Analyses

Parameter	Sites						
	Gate 25	Site A	Site B	Site C	Site D	Site E	Site F
Temperature, DO, Conductivity, pH, Turbidity, Fecal Coliforms, *E. coli*	10	9	9	10	9	10	7
TOC, DOC, Particle Counts	7	7	7	7	6	7	7
UV_{254}	6	6	6	6	5	6	6

The ANOVA analyses by site are presented in Appendix B, and Table 16 lists the P-values for each of the water quality parameters according to variation by site. The two water quality parameters that were found to be statistically different by site (P-value ≤ 0.05) are conductivity and pH. All of the other water quality parameters were not statistically different by site. The different surrounding materials and land uses at each site most likely factor into the variation in the two water quality parameters by sampling location. Gate 25 had the highest conductivity levels with an average of 481 µS, and Site E had the lowest with an average of 139 µS. Site D had the highest pH levels with an average of 7.3 and Site C had the lowest with an average of 6.8.

One water quality parameter that was expected to be statistically different by site was dissolved oxygen. The flow characteristics at each site were expected to cause site variations in dissolved oxygen levels, but this result was not found.

Fecal coliforms and *E. coli* were found to not be statistically different by site. This result ruled out any one site being specified as a source of biological contamination. The biological contamination seems to be spread throughout the entire watershed subbasin.

Table 16: P-Values for Each Water Quality Parameter According to Variations by Site

Water Quality Parameter	P-Value
Conductivity	3.37×10^{-5}
pH	1.26×10^{-3}
Dissolved Oxygen	0.125
Particle Count	0.146
Turbidity	0.179
UV_{254}	0.332
Fecal Coliforms	0.301
E. coli	0.447
DOC	0.686
TOC	0.693
Temperature	0.987

4.5.2.2 ANOVA Seasonal Analysis

Differences in the eleven water quality parameters were analyzed to determine seasonal variations. There are a different number of data points for each of the water quality parameters and each of the seasons, as shown in Table 17. The data points are totaled from the 3 winter, 2 spring, 3 summer and 2 fall sampling events. The ANOVA analysis for TOC, DOC, UV_{254} and particle counts is based on only 3 seasons.

Table 17: Number of Data Points for the ANOVA Seasonal Analyses

Parameter	Season			
	Winter	Spring	Summer	Fall
Temperature, DO, Conductivity, pH, Turbidity, Fecal Coliforms, *E. coli*	18	12	20	14
TOC, DOC, Particle Counts	14	0	20	14
UV_{254}	14	0	19	14

The ANOVA results for the seasonal analyses are presented in Appendix C and summarized in Table 18 which lists the P-values for each of the water quality parameters according to variation by season. All of the water quality parameters were found to be statistically different by season. Several water quality parameters were expected to be statistically different by season. Due to seasonal temperature changes in New England, it was expected that temperature would be statistically different by season as well as dissolved oxygen which is inversely correlated to temperature based on saturation values of dissolved oxygen at different temperatures.

Table 18: P-Values for Each Water Quality Parameter According to Variation by Season

Water Quality Parameter	P-Value
Temperature	4.15×10^{-24}
TOC	2.80×10^{-16}
DOC	3.67×10^{-15}
Dissolved Oxygen	1.84×10^{-12}
pH	7.16×10^{-4}
UV_{254}	2.00×10^{-4}
Conductivity	1.89×10^{-3}
Turbidity	7.11×10^{-3}
Particle Count	0.016
E. coli	0.018
Fecal Coliforms	0.023

It was also expected that fecal coliform levels and *E. coli* levels would be statistically different by season. Temperature affects the survival of these microorganisms; thus concentrations tend to be higher in warmer verses colder temperatures due to increased survival times. Fecal coliform levels averaged 165 cfu/100 ml in the winter months verses an average of 301 cfu/100 ml in the summer months. A seasonal trend in microorganisms that was not expected was the high average levels in the spring and fall which were 684 cfu/100 ml and 847 cfu/100 ml, respectively.

Organic matter measurements (TOC, DOC and UV_{254}) all differed by season as well. It was expected that organic matter may differ by season due to additional organic matter entering the water in the fall season from the large amount of leaves that fall into the brook. This theory seemed accurate as the fall sampling events had the highest average TOC value of 6.6 mg/l, verses an average of 3.2 mg/l in the winter and an average of 2.4 mg/l in the summer.

Turbidity and particle counts varied by season as well. It was expected that they may vary by season due to high flows in the spring causing erosion, thus increasing turbidity and particle counts. It was also expected that leaves entering the brook in the fall may increase turbidity and particle counts. Turbidity was high in the spring, but not in the fall. The average turbidity in the spring was 4.5 ntu, while the next highest average turbidity was 2.1 ntu in the summer months. Particle counts were highest in the fall with an average count of 3725 /ml. Average counts in the winter and summer were 3535/ml and 2301/ml, respectively.

pH and conductivity differed by season as well. The variation by season shows the difference in material entering the brook throughout the year, which could be caused by a number of factors. One example would be a large amount of organic matter entering the brook in the fall causing lower conductivity measurements.

4.5.3 ANOVA Analysis of the Binary Data Set

Binary data sets were created for several of the water quality parameters for the purpose of further ANOVA analysis to assess pollution levels in the brook. The binary data set was created by setting a bench mark level for several of the water quality parameters. Any data point in the data set that was greater than the specified bench mark level was considered to be "polluted" and any value less than the specified bench mark levels was considered to be "not polluted".

Binary data sets were created for the following parameters: conductivity, turbidity, fecal coliforms, *E. coli*, and SUVA. Benchmark levels are shown in Table 19. Water quality parameters such as temperature, pH, and dissolved oxygen were not included in the binary data sets due to the lack of good benchmark levels for these

parameters. In this research, the temperature, pH and dissolved oxygen were all in ranges normal for surface waters and would therefore not be classified as "polluted".

The conductivity benchmarks of 150 and 500 µS are based upon the EPA's definition of a healthy freshwater fishery (EPA, 2005e). The turbidity benchmark of 5 ntu is based upon the turbidity requirement to avoid filtration of a surface water source for a public water supply (EPA, 1991). The SUVA benchmark is based upon the EPA's required level of organic carbon removal to reduce disinfection byproducts in drinking water treatment plants. SUVA can be calculated using Equation 1 (EPA, 2006b). The fecal coliform and *E. coli* levels are based upon the EPA's guidelines for safe body contact with a water (EPA, 2006a).

$$SUVA = \frac{UV_{254}\left(cm^{-1}\right)}{DOC\left(mg/L\right)} * 100 \qquad \textbf{(Equation 1)}$$

Table 19: Benchmark Pollution Levels

Water Quality Parameter	Benchmark Levels	Source
Conductivity	> 150 µS < 500 µS	EPA "freshwater fishery"
Turbidity	< 5 ntu	EPA "filtration avoidance"
Fecal Coliforms	< 200 cfu/100 ml	EPA "safe to swim"
E. coli	< 126 cfu/100 ml	EPA "safe to swim"
SUVA	< 2 L/mg-m	EPA "avoid TOC removal"

4.5.3.1 ANOVA Analysis of the Binary Data Set by Site

The ANOVA binary data set site analysis was carried in the same manner and with the same number of data points as the ANOVA site analysis in Section 4.5.2.1. Table 20 lists the P-values for each of the 5 water quality parameters assessed. Conductivity and turbidity were the two water quality parameters that were found to vary by site. Conductivity was found to vary by site with an ANOVA analysis of both the original data set and the binary data set. Turbidity, which was previously found to not

statistically vary by site, was found to vary by site with an ANOVA analysis of a binary data set.

Based on the binary data set of "polluted" or "not polluted", both conductivity and turbidity varied by site. In terms of turbidity, Site C was the most polluted with polluted turbidity levels occurring on 2 out of 10 sampling events. Other polluted sites in terms of turbidity were Sites D (polluted 1 out of 10 sampling events) and Site A (polluted 1 out of 9 sampling events). The other sites were never classified as polluted in terms of turbidity.

In terms of conductivity, Site E was the most polluted. The conductivity fell below the 150 µS benchmark on 7 out of 10 sampling events. The next highest polluted site in terms of conductivity was Gate 25, which fell above the 500 µS benchmark on 4 out of 10 sampling events.

The binary analysis was unable to classify sites as polluted or not polluted in terms of indicator organisms. No one specific location can be specified as a pollution source or a polluted area in terms of indicator organisms.

Table 20: P-Values for each Binary Water Quality Parameter According to Site Variation

Water Quality Parameter	P-Value
Conductivity	3.30×10^{-3}
Turbidity	0.0408
Fecal Coliforms	0.550
SUVA	0.574
E. coli	0.656

4.5.3.2 ANOVA Analysis of the Binary Data Set by Season

The ANOVA binary data set site analysis was carried in the same manner and with the same number of data points as the ANOVA seasonal analysis in Section 4.5.2.2. Table 21 lists the P-values for each of the 5 water quality parameters assessed. With the binary data set, fecal coliform levels were the only water quality parameter found to be

statistically different from season to season, while the original ANOVA analysis found all 5 water quality parameters to statistically differ due to seasonal changes. The West Boylston Brook was most polluted in terms of fecal coliforms in the spring (7 out of 12 data points classified as polluted) and the fall (11 out of 14 data points classified as polluted). In the winter, the brook was classified as polluted for 11 out of 20 data points, and in the summer only 4 out of 18 data points were considered polluted.

Table 21: P-Values for each Binary Water Quality Parameter According to Seasonal Variation

Water Quality Parameter	P-Value
Fecal Coliforms	0.012
E. coli	0.051
Turbidity	0.191
Conductivity	0.196
SUVA	0.325

5.0 Conclusions and Recommendations

Conclusions and recommendations were made based on the culmination of the water quality parameters analyses. This chapter first presents conclusion on the water quality data, statistical analyses, and surrounding land uses. Secondly, the chapter provides recommendations for improving water quality and for future water quality monitoring in the WBB subbasin.

5.1 Conclusions

Microbiological contamination in the West Boylston Brook seemed to be a result of development in the subbasin, but no specific source could be identified from this study. Neither fecal coliforms nor *E. coli* were statistically different by site when the raw data was analyzed. The same result was obtained through the binary number system analysis. The high levels of biological indicator organisms seem to be entering the brook throughout the entire subbasin because the only site with consistently low levels of biological indicator organisms was Site E. Even though the sites were not statistically different, Site E tended to have comparably low levels of fecal coliforms and *E. coli*. Other than the protected land upstream at Site E, all other locations had relatively high fecal coliform and *E. coli* contamination problems.

The water quality parameters that were statistically different by site were pH and conductivity. All of the water quality parameters were statistically different by season. As conductivity was the most sensitive water quality parameter by site, and thus may be the best tool for measuring human influence on the brook. Gate 25 had the highest average conductivity measurement which is the most downstream location that is heavily influenced by upstream development and land uses. Site E, which is an upstream location with little to no human influence, had the lowest average conductivity measurement. The trend in conductivity was that the more downstream locations tended to have higher conductivity levels. Even though conductivity may be the best parameter to indicate human influences on the brook, conductivity was also statistically different by season and by stage height, so these variations would have to be accounted for.

5.2 Recommendations

Site C, which periodically had high levels of fecal coliforms and *E. coli,* may be a source of microbial contamination. This site was influenced by groundwater recharge (based upon seasonal temperature trends) and had consistently high levels of fecal coliforms and *E. coli*. The fecal coliform and *E. coli* levels were not lower in the winter months as was found at the other six sampling locations. The high number of biological indicator organisms in the winter months may be due to contaminated groundwater entering the brook or may be due to the indicator organisms surviving longer in the warmer environment at this site. Further analysis should be done on the water quality in this area and septic systems in the area should be inspected.

Many of the water quality parameters tested proved to be redundant. All three measurement of organic carbon (TOC, DOC, and UV_{254}) followed similar trends. Also, the two biological indicator organisms, fecal coliforms and *E. coli,* followed similar trends. For future monitoring, it is probably only necessary to measure one of the organic carbon parameters and one of the biological indicator organisms.

For future water quality monitoring, all of the water quality parameters were found to be statistically different by season, and therefore any water quality monitoring of the brook would have to be done over multiple seasons.

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Appendix A: Experimental Results

		Field measurements			Lab measurements - physical/chemical						Lab measurements - microbiological				
Site	**Date**	**°C**	**mg/L**	**mS**		**cm-1**	**mg/L**	**mg/L**	**ntu**	**#/mL**	**cfu/100 mL**	**cfu/100 mL**	**cfu/100 mL**	**cfu/100 mL**	**cfu/100 mL**
		Temp	**DO**	**Conduct.**	**pH**	**UV254**	**TOC**	**DOC**	**turbidity**	**particles**	**FC**	**E. coli**	**Rhodo**	**Bifido**	**Phage**
Gate 25	02/23/05	2.5	12.89	536	7.22				1.67		58	50	20050	<2	<1
Site A	02/23/05	not sampled													
Site B	02/23/05	not sampled													
Site C	02/23/05	5	10.6	803	7.07				1.04		7	2	6980	2 (var ferm)	<1
Site D	02/23/05	1.1	12.76	189.2	7.41				2.37		64	64	10000	16 (var ferm)	<1
Site E	02/23/05	1.5	11.24	103.3	7.28				0.80		8	8	6200	<2	<1
Site F	02/23/05	not sampled													
Gate 25	03/30/05	4.6	12.57	371.9	6.90				3.38		35	35	17800	<2	0.5
Site A	03/30/05	4.9	12.18	333.5	6.97				2.63		30	30	23600	<2	<1
Site B	03/30/05	4.9	12.34	317.0	7.02				1.94		31	31	24800	<2	0.5
Site C	03/30/05	6.3	11.15	430.0	7.00				17.20		410	410	26800	95	<1
Site D	03/30/05	5.0	11.69	287.0	7.06				2.38		30	30	19800	<2	1.0
Site E	03/30/05	2.8	11.67	123.0	7.02				1.66		14	14	21800	<2	<1
Site F	03/30/05	not sampled													
Gate 25	05/24/05	9.4	10.62	300.2	6.8				3.05		764	764	312800	<2	<1
Site A	05/24/05	9.4	10.72	353	6.99				2.10		636	636	183200	<2	<1
Site B	05/24/05	9.4	11.77	356.8	7.04				1.92		490	490	76200	<2	<1
Site C	05/24/05	9.1	10.55	249	6.79				11.50		4800	4800		<2	<1
Site D	05/24/05	9.8	11.36	273	6.78				2.76		520	520	98800	<2	<1
Site E	05/24/05	11.8	10.5	194	6.85				2.01		450	450	7200	<2	<1
Site F	05/24/05	not sampled													
Gate 25	06/22/05	12.5	10.06	855	7.36	0.043	1.28	1.28	0.72	1327	387	80	2120	<2	<1
Site A	06/22/05	12.8	9.62	587	7.05	0.034	2.31	1.29	5.10	1997	144	67	14920	<2	<1
Site B	06/22/05	12.6	9.94	660	6.99	0.030	1.29	1.30	0.21	910	54	36	2420	<2	<1
Site C	06/22/05	11.8	8.26	520	6.54	0.021	1.04	1.03	1.18	1410	191	88	6860	<2	<1
Site D	06/22/05	17.2	9.92	336.7	7.61	0.113	3.45	3.43	8.37	8843	299	180	14300	<2	<1

Site E	06/22/05	18.6	8.76	206.8	7.23	0.152	3.97	3.81	1.86	3314	35	32	2550	<2	<1
Site F	06/22/05	15.5	10.34	255.5	7.26	0.079	2.38	2.33	2.13	2117	193	117			
Gate 25	07/20/05	15	8.14	630	7.31	0.043	1.87	1.42	2.98	2822	224	67	22600	<2	<1
Site A	07/20/05	14.9	7.7	333	7.18	0.029	2.38	1.70	1.85	1599	96	59	6200	<2	1.0
Site B	07/20/05	14.7	7.95	496	7.12	0.024	1.38	1.34	0.27	861	146	102		<2	<1
Site C	07/20/05	13.8	6.35	401	6.57	0.022	1.26	0.97	2.05	1678	99	52		<2	<1
Site D	07/20/05	21.2	7.52	398	7.6	0.141	4.01	3.95	1.17	3132	271	155		<2	<1
Site E	07/20/05	22.4	5.94	205	7.18	0.183	5.49	5.12	3.01	4948	85	26		<2	<1
Site F	07/20/05	19.5	7.41	284	7.53	0.091	2.85	2.65	2.24	1495	377	233			
Gate 25	08/22/05	15	9.25	790	7.36	0.042	1.32	1.39	0.35	1032	280	103		<2	<1
Site A	08/22/05	15.6	9.12	590	7.26	0.032	2.01	1.44	1.49	1520	303	133		<2	<1
Site B	08/22/05	15.7	8.55	523	6.9	0.028	1.34	1.56	0.42	478	340	127		<2	0.5
Site C	08/22/05	15.2	7.3	409	6.44	0.024	1.84	1.83	0.68	1211	1109	300		<2	<1
Site D	08/22/05	Not sampled												<2	<1
Site E	08/22/05	18.9	8.08	144	7.31	0.114	4.06	3.89	1.89	3844	370	213		<2	<1
Site F	08/22/05	17.2	9.63	173	6.99	0.068	2.05	1.96	3.46	1484	1027	191			
Gate 25	10/23/05	9.3	11.2	170	7.39	0.215	6.55	6.42	1.72	4524	1322	861			
Site A	10/23/05	9.1	10.9	162	7.15	0.216	6.78	6.48	1.64	4582	1217	728			
Site B	10/23/05	9.1	11.0	165	7.20	0.212	6.64	6.16	1.54	4716	1311	744			
Site C	10/23/05	9.8	10.0	110	6.71	0.229	7.91	7.53	3.18	6583	1000	740			
Site D	10/23/05	9.0	10.0	143	6.99	0.225	6.89	6.57	1.35	4754	1213	573			
Site E	10/23/05	9.0	9.9	116	6.95	0.164	5.27	4.75	1.16	4710	42	42			
Site F	10/23/05	9.3	10.3	203	6.90	0.167	5.47	5.37	1.36	3228	500	447			
Gate 25	11/29/05	6.9	10.1	426	7.23	0.079	6.62	5.53	0.75	2354	440	427		<2	1
Site A	11/29/05	6.7	9.4	228	7.18	0.077	7.28	5.40	2.47	2760	520	493		<2	<1
Site B	11/29/05	6.4	9.6	213	7.18	0.076	6.111	5.641	0.64	2060	420	380		<2	<1
Site C	11/29/05	9	7.4	390	6.72	0.038	5.095	4.671	0.67	1483	121	96		31 (ferm)	<1
Site D	11/29/05	5	9.5	169	7.36	0.098	7.921	5.677	0.91	3183	1000	907		<2	1

Site E	11/29/05	4	8.94	107.4	6.98	0.1335	7.872	6.651	1.6	4378.905	97.33	92		<2	0.5
Site F	11/29/05	5.5	9.24	194	7.16	0.062	6.49	4.699	1.445	2837	2667	2667			0
Gate 25	01/24/06	2.9	13.4	265	7.09	#VALUE!	3.336	2.395	0.8625	2036	177	121			
Site A	01/24/06	3	12	227.6	7.18	#VALUE!	3.194	2.374	2.965	4020	175	136			
Site B	01/24/06	2.6	12.06	214.2	7.26	#VALUE!	3.384	2.055	0.7805	3033	231	156			
Site C	01/24/06	6	9.72	369.3	7.05	#VALUE!	2.769	2.023	1.38	4638	487	344			
Site D	01/24/06	0.9	12.57	181.5	7.21	#VALUE!	3.42	2.791	0.7745	3402	369	247			
Site E	01/24/06	1.9	10.83	95.3	7.12	#VALUE!	3.214	2.411	0.86	2521	141	133			
Site F	01/24/06	2.7	11.49	175.8	7.29	#VALUE!	2.987	1.875	0.595	4009	141	98			
Gate 25	02/18/06	2.2	14.04	462.6	7.49	0.048	2.312	2.073	1.06	3799	61	62			
Site A	02/18/06	2.1	12.9	379	7.57	0.041	2.602	2.142	1.94	5164	105	92			
Site B	02/18/06	1.7	11.72	198.4	7.88	0.046	2.487	1.957	0.8925	3684	68	59			
Site C	02/18/06	4.9	10.05	492.2	7.05	0.019	2.043	1.339	1.245	3597	787	727			
Site D	02/18/06	1.5	11.39	154.8	7.71	0.055	2.931	2.086	1.1	3747.68	77.6	70.67			
Site E	02/18/06	1.7	10.25	96	7.6	0.070	3.065	2.3	1.048	2931.46	11.03	10.67			
Site F	02/18/06	2.6	11.38	152.4	7.72	0.045	2.539	1.936	0.897	2903.55	8.27	6.67			

Appendix B: ANOVA Analyses by Site

Anova: Single Factor
Temperature
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	72.3	10.32857	50.06238
Site E	10	92.6	9.26	66.69822
Site D	9	70.7	7.855556	52.65028
Site C	10	90.9	9.09	13.221
Site B	9	77.1	8.566667	25.83
Site A	9	78.5	8.722222	24.74944
Gate 25	10	80.3	8.03	24.95122

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	33.80394	6	5.633989	0.155135	0.987251	2.262531
Within Groups	2070.046	57	36.3166			
Total	2103.85	63				

Anova: Single Factor
Dissolved Oxygen
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	69.77	9.967143	1.95559
Site E	10	96.07	9.607	2.959357
Site D	9	96.68	10.74222	2.786494
Site C	10	91.42	9.142	2.789551
Site B	9	94.89	10.54333	2.5856
Site A	9	94.57	10.50778	2.837994
Gate 25	10	112.27	11.227	3.743134

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	30.06776	6	5.011294	1.754109	0.12514	2.262531
Within Groups	162.8426	57	2.856888			
Total	192.9104	63				

Anova: Single Factor
Conductivity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	1437.2	205.3143	2260.781
Site E	10	1390.5	139.05	2092.605
Site D	9	2132	236.8889	8160.226
Site C	10	4173.4	417.34	32383.87
Site B	9	3143.3	349.2556	30365.11
Site A	9	3192.6	354.7333	22538.83
Gate 25	10	4806.4	480.64	50216.31

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	852375.2	6	142062.5	6.404715	3.37E-05	2.262531
Within Groups	1264313	57	22180.93			
Total	2116688	63				

Anova: Single Factor
pH
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	50.85	7.264286	0.082962
Site E	10	71.52	7.152	0.047662
Site D	9	65.73	7.303333	0.10025
Site C	10	67.94	6.794	0.055804
Site B	9	64.59	7.176667	0.0825
Site A	9	64.53	7.17	0.03195
Gate 25	10	72.15	7.215	0.049406

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.621365	6	0.270227	4.28906	0.001236	2.262531
Within Groups	3.591221	57	0.063004			
Total	5.212586	63				

Anova: Single Factor
UV
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	6	0.5122	0.085367	0.001853
Site E	6	0.8155	0.135917	0.001612
Site D	5	0.6316	0.12632	0.004017
Site C	6	0.3524	0.058733	0.007041
Site B	6	0.41625	0.069375	0.005242
Site A	6	0.4287	0.07145	0.005299
Gate 25	6	0.47055	0.078425	0.004688

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.030565	6	0.005094	1.196565	0.331749	2.380311
Within Groups	0.144748	34	0.004257			
Total	0.175313	40				

Anova: Single Factor
TOC
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	25.216	3.602286	2.84436
Site E	7	33.0965	4.728071	2.727464
Site D	6	29.1035	4.850583	4.068678
Site C	7	22.676	3.239429	6.068962
Site B	7	23.526	3.360857	5.10591
Site A	7	27.1435	3.877643	4.845314
Gate 25	7	24.3025	3.471786	5.231289

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17.12323	6	2.853872	0.645447	0.693331	2.32977
Within Groups	181.2832	41	4.421541			
Total	198.4064	47				

Anova: Single Factor
DOC
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	20.7545	2.964929	2.109171
Site E	7	29.041	4.148714	2.298536
Site D	6	25.2035	4.200583	2.482807
Site C	7	20.0725	2.8675	5.737435
Site B	7	20.1075	2.8725	4.394063
Site A	7	21.048	3.006857	4.293468
Gate 25	7	20.8235	2.974786	4.480776

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.58705	6	2.431175	0.654508	0.686299	2.32977
Within Groups	152.2947	41	3.714505			
Total	166.8818	47				

Anova: Single Factor
Turbidity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	12.112	1.730286	0.931152
Site E	10	15.888	1.5888	0.443411
Site D	9	21.1745	2.352722	5.617785
Site C	10	40.117	4.0117	32.03141
Site B	9	8.602	0.955778	0.462836
Site A	9	22.18	2.464444	1.204847
Gate 25	10	16.5315	1.65315	1.231234

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	55.59022	6	9.265036	1.438102	0.216249	2.262531
Within Groups	367.2252	57	6.442547			
Total	422.8154	63				

Anova: Single Factor
Particles
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	18073.61	2581.944	871249.2
Site E	7	26648.18	3806.882	851346.6
Site D	6	27061.02	4510.169	4861006
Site C	7	20598.38	2942.625	4272618
Site B	7	15740.96	2248.709	2608218
Site A	7	21641.71	3091.672	2230845
Gate 25	7	17894.27	2556.324	1605707

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	24596568	6	4099428	1.698688	0.145819	2.32977
Within Groups	98944938	41	2413291			
Total	1.24E+08	47				

Anova: Single Factor
Fecal Coliforms
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	4912.61	701.8014	861235.9
Site E	10	1254.3	125.43	24683.02
Site D	9	3843.82	427.0911	176356
Site C	10	9010.37	901.037	2026228
Site B	9	3091.223	343.4692	159075
Site A	9	3226.034	358.4482	145443
Gate 25	10	3748.516	374.8516	159778.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3769113	6	628185.6	1.238527	0.300544	2.262531
Within Groups	28910614	57	507203.7			
Total	32679727	63				

Anova: Single Factor

E. coli

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	3759.11	537.0157	901011.5
Site E	10	1021.61	102.161	19290.01
Site D	9	2746.34	305.1489	88797.06
Site C	10	7559.11	755.911	2088671
Site B	9	2125.91	236.2122	61648.46
Site A	9	2374.474	263.8304	75632.19
Gate 25	10	2569.616	256.9616	98908.58

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2793796	6	465632.7	0.980224	0.447136	2.262531
Within Groups	27076520	57	475026.7			
Total	29870316	63				

Appendix C: ANOVA Analyses by Season

Anova: Single Factor
Temperature
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	87.4	7.283333	8.028788
Summer	20	320.1	16.005	8.466816
Winter	18	46.8	2.6	1.938824
Fall	14	108.1	7.721429	3.666429

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1774.04	3	591.3468	107.5796	4.15E-24	2.758078
Within Groups	329.8097	60	5.496829			
Total	2103.85	63				

Anova: Single Factor
DO
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	137.12	11.42667	0.528515
Summer	20	169.84	8.492	1.554048
Winter	18	211.29	11.73833	1.438956
Fall	14	137.42	9.815714	0.936196

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	120.937	3	40.31234	33.60604	7.14E-13	2.758078
Within Groups	71.97338	60	1.199556			
Total	192.9104	63				

Anova: Single Factor
Conductivity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	3588.4	299.0333	6869.902
Summer	20	8797	439.85	41737.52
Winter	18	5095.6	283.0889	35820.8
Fall	14	2794.4	199.6	9213.942

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	519371.6	3	173123.9	6.503051	0.000697	2.758078
Within Groups	1597317	60	26621.95			
Total	2116688	63				

Anova: Single Factor
pH
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	83.22	6.935	0.011027
Summer	20	142.79	7.1395	0.1087
Winter	18	132.2	7.344444	0.067167
Fall	14	99.1	7.078571	0.044352

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.307575	3	0.435858	6.696909	0.000566	2.758078
Within Groups	3.905011	60	0.065084			
Total	5.212586	63				

Anova: Single Factor
UV
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Summer	20	1.3119	0.065595	0.002493
Winter	7	0.32425	0.046321	0.000236
Fall	14	1.99105	0.142218	0.004861

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.063344	2	0.031672	10.74894	0.0002	3.244821
Within Groups	0.111969	38	0.002947			
Total	0.175313	40				

Anova: Single Factor
TOC
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Summer	20	47.573	2.37865	1.502189
Winter	14	44.608	3.186286	0.050892
Fall	14	92.883	6.6345	0.860913

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	158.0114	2	79.00568	88.01214	2.8E-16	3.20432
Within Groups	40.39506	45	0.897668			
Total	198.4064	47				

Anova: Single Factor
DOC
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Summer	20	43.6685	2.183425	1.45332
Winter	14	31.848	2.274857	0.089363
Fall	14	81.534	5.823857	0.71687

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	128.7877	2	64.39384	76.06749	3.67E-15	3.20432
Within Groups	38.0941	45	0.846536			
Total	166.8818	47				

Anova: Single Factor
Turbidity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	52.53	4.3775	23.42922
Summer	20	41.375	2.06875	3.726734
Winter	18	22.28	1.237778	0.390505
Fall	14	20.42	1.458571	0.486005

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	81.32935	3	27.10978	4.763261	0.004809	2.758078
Within Groups	341.486	60	5.691434			
Total	422.8154	63				

Anova: Single Factor
Particles
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Summer	20	46019.67	2300.984	3630748
Winter	14	49486.4	3534.743	675180
Fall	14	52152.04	3725.145	1917020

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20858695	2	10429348	4.570586	0.015592	3.20432
Within Groups	1.03E+08	45	2281840			
Total	1.24E+08	47				

Anova: Single Factor
Fecal Coliforms
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	8210	684.1667	1754216
Summer	20	6030	301.5	81071.32
Winter	18	2975.903	165.3279	40480.8
Fall	14	11870.97	847.9264	490887.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4773291	3	1591097	3.420925	0.022793	2.758078
Within Groups	27906436	60	465107.3			
Total	32679727	63				

Anova: Single Factor

E. coli

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	8210	684.1667	1754216
Summer	20	2361	118.05	5508.997
Winter	18	2388.19	132.6772	29604.67
Fall	14	9196.98	656.9271	413993.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4584081	3	1528027	3.625752	0.017916	2.758078
Within Groups	25286235	60	421437.3			
Total	29870316	63				

Appendix D: Binary ANOVA Analysis by Site

Anova: Single Factor
Conductivity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	0	0	0
Site E	10	7	0.7	0.233333
Site D	10	1	0.1	0.1
Site C	10	3	0.3	0.233333
Site B	9	2	0.222222	0.194444
Site A	9	0	0	0
Gate 25	10	4	0.4	0.266667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.498291	6	0.583048	3.734372	0.003297	2.259605
Within Groups	9.055556	58	0.15613			
Total	12.55385	64				

Anova: Single Factor
Turbidity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	0	0	0
Site E	10	0	0	0
Site D	10	1	0.1	0.1
Site C	10	2	0.2	0.177778
Site B	9	0	0	0
Site A	9	1	0.111111	0.111111
Gate 25	10	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.364957	6	0.060826	1.041026	0.408489	2.259604
Within Groups	3.388889	58	0.058429			
Total	3.753846	64				

Anova: Single Factor
Fecal Coliforms
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	4	0.571429	0.285714
Site E	10	2	0.2	0.177778
Site D	10	6	0.6	0.266667
Site C	10	6	0.6	0.266667
Site B	9	5	0.555556	0.277778
Site A	9	4	0.444444	0.277778
Gate 25	10	6	0.6	0.266667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.287424	6	0.214571	0.831962	0.550152	2.259604
Within Groups	14.95873	58	0.257909			
Total	16.24615	64				

Anova: Single Factor
E. coli
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	7	4	0.571429	0.285714
Site E	10	3	0.3	0.233333
Site D	10	6	0.6	0.266667
Site C	10	6	0.6	0.266667
Site B	9	5	0.555556	0.277778
Site A	9	5	0.555556	0.277778
Gate 25	10	3	0.3	0.233333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.087424	6	0.181237	0.693446	0.655786	2.259604
Within Groups	15.15873	58	0.261357			
Total	16.24615	64				

Anova: Single Factor

SUVA

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Site F	8	5	0.625	0.267857
Site E	8	6	0.75	0.214286
Site D	8	3	0.375	0.267857
Site C	8	3	0.375	0.267857
Site B	8	3	0.375	0.267857
Site A	8	3	0.375	0.267857
Gate 25	8	5	0.625	0.267857

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.25	6	0.208333	0.800654	0.574166	2.290435
Within Groups	12.75	49	0.260204			
Total	14	55				

Appendix D: Binary ANOVA Analysis by Season

Anova: Single Factor
Conductivity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	1	0.083333	0.083333
Summer	20	9	0.45	0.260526
Winter	18	6	0.333333	0.235294
Fall	14	4	0.285714	0.21978

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.02619	3	0.342063	1.613024	0.195783	2.758078
Within Groups	12.72381	60	0.212063			
Total	13.75	63				

Anova: Single Factor
Turbidity
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	2	0.166667	0.151515
Summer	20	2	0.1	0.094737
Winter	18	0	0	0
Fall	14	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.283333	3	0.094444	1.634615	0.190844	2.758078
Within Groups	3.466667	60	0.057778			
Total	3.75	63				

Anova: Single Factor
Fecal Coliforms
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	7	0.583333	0.265152
Summer	20	11	0.55	0.260526
Winter	18	4	0.222222	0.183007
Fall	14	11	0.785714	0.181319

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.649454	3	0.883151	3.973708	0.011932	2.758078
Within Groups	13.33492	60	0.222249			
Total	15.98438	63				

Anova: Single Factor
E. coli
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	7	0.583333	0.265152
Summer	20	8	0.4	0.252632
Winter	18	6	0.333333	0.235294
Fall	14	11	0.785714	0.181319

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.92619	3	0.642063	2.737269	0.051255	2.758078
Within Groups	14.07381	60	0.234563			
Total	16	63				

Anova: Single Factor
SUVA
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Summer	20	16	0.8	0.168421
Winter	7	5	0.714286	0.238095
Fall	14	8	0.571429	0.263736

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.430662	2	0.215331	1.015568	0.371816	3.244821
Within Groups	8.057143	38	0.21203			
Total	8.487805	40				